Neurons, receptors and channels

David A. Brown

University College London, London WC1E 6BT, UK. Email d.a.brown@ucl.ac.uk

Key words: sympathetic ganglion; brain slice; GABA; acetylcholine; muscarinic receptors; M-current

Abstract I recount some adventures I and my colleagues have had over some 60 years from 1957 studying the effects of drugs and neurotransmitters on neuronal excitability and ion channel function largely but not exclusively using sympathetic neurons as test objects. Studies include: effects of centrally-active drugs on sympathetic transmission; neuronal action and neuroglial uptake of GABA in ganglia and brain; action of muscarinic agonists on sympathetic neurons; action of bradykinin on neuroblastoma-derived cell ; identification of M-current as a target for muscarinic action, and experiments to determine its distribution, molecular composition, neurotransmitter sensitivity and intracellular regulation by phospholipids and their hydrolysis products. Techniques used include electrophysiological recording (extracellular, intracellular microelectrode, whole-cell and single channel patch-clamp), autoradiography, mRNA and cDNA expression, antibody injection, antisense knockdown, and membrane=targeted lipidated peptides. I finish with some recollections about my scientific career, funding, and changes in laboratory life and pharmacology research over 60 years. [149 words]

To my knowledge there were no scientists in my ancestry. My father (born 1900, of part-Scottish extraction) left school at 14 to become a messenger boy and postman, and my mother (born 1899, of part-Irish ancestry) was a waitress and wartime factory worker. I was born in London and attended the local state primary school during and after the second World War (from 1940 to 1947). Class sizes were large (up to 40 to 45 pupils} since teachers were in short supply. At the end of primary school, with a push from my parents and the help of my primary school Headmaster (Mr. Kitchen), I gained a scholarship to Haberdashers Aske's Hampstead School, a 'direct grant' school – that is, a private day-school but with a proportion of pupils paid for from public funds.

I spent an enjoyable 7 years at Habs, eventually concentrating on sciences. I then faced a conundrum: the school wanted to enter me for some Cambridge University examinations but that meant staying at school for an extra year. However, this would have imposed an additional burden on my relatively-poor parents, so instead I wrote to my 'local' University, University College London (UCL), enquiring about courses that included chemistry and zoology. UCL invited me to an interview and subsequently (1954) enrolled me for a two-year General Degree in Chemistry, Physiology and Zoology.

The course was fairly hard-going because each subject was regarded as a 'main' subject. Chemistry was dominant in terms of time consumption with extensive practicals; Physiology tuition was largely tagged onto the medical student's course with additional tutorials and practicals; and Zoology followed aspects of the 3-year BSc Zoology course. Having gained an honours grade in the two-year General degree, I was entitled to continue at UCL for a third year, and hence enrolled for the final year of the Special Physiology BSc. For this we were joined with some intercalating medical students. Overall, at that time, I did not really feel 'at home' with physiology as a main subject, partly because of my poor knowledge of anatomy and 'integrated' physiology, and partly the almost-encyclopaedic approach of our standard textbook (Starling's "*Textbook of Physiology*"). My interest was restored by discovering Davson's "*Textbook of General Physiology*" which provided a superb link between my previous chemistry courses (particularly physical chemistry, which had concentrated on thermodynamics) and biology; and then by Hugh Davson's own course on ocular and cerebrospinal fluids. At the end of the degree course Davson pointed me toward Professor Peter Quilliam (a previous scientific colleague of Davson's, and at that time Head of Pharmacology at St. Bartholomew's Hospital Medical College, 'Barts') for a possible postgraduate studentship in Pharmacology, starting in September 1957.

Although an obvious link between chemistry and physiology, I had not studied pharmacology at UCL because this was restricted to medical students. I tried to repair this deficit in two ways. First, one of my ex-student colleagues, Alan Boura, had by now left UCL after his general degree and was back at the Wellcome Laboratories, and he arranged for me to work as a temporary technician at the Wellcome Labs that summer (1957). Second, I bought a secondhand copy of Dick Barlow's "*Chemical Pharmacology*" which I read during the long trans-London journey to Beckenham and back. I can't remember much about my own work at Wellcome now; more vividly I recall being an onlooker at some early experiments of Alan's on the new sympathetic blocking drug bretylium.

Come September 1957 I duly turned up at Barts' Pharmacology Department. Barts was a very old hospital (founded in 1123), located south of Smithfield meat market in central London but the preclinical medical school was in a then-modern building in Charterhouse Square, north of Smithfield meat market, The Pharmacology Department was quite small, with Peter Quilliam (usually called "Q" by the staff) as Professor and departmental head, Don Mason (from May & Baker pharmaceutical company) as senior lecturer, Peter Bell as electronics engineer, and Brian Prichard (a clinical graduate, later Professor of Clinical Pharmacology at UCL) as a Research Fellow. Contemporary PhD students included *inter alia* Brian Payton (a medical graduate and a good friend, later a Faculty Professor at the Memorial University of Newfoundland), and Keith Matthews (later Reader in Pharmacology at Cambridge University).

On first meeting, two of Q's comments entered my long-term memory – that our working hours were 8.30 to 6.30, and that I was no longer at UCL so should wear a jacket and tie, since

we'll be meeting medical students (Q himself was always formally dressed in grey suit and tie, allegedly his ex-WWII 'demob' suit) . Teaching was (correctly) taken very seriously, every Tuesday afternoon being devoted to medical student teaching with a lecture (usually by Q or Don Mason), sometimes followed by a live demonstration, and then a class practical. For the latter, the 100-odd medical students were divided into 10 groups and followed a rotation of 10 practical experiments. Each student was provided with a loose-leaf book [**1**] describing the schedules for these experiments, along with spaces for describe results and answer questions. All graduate students were enrolled as demonstrators for these practicals, so their first job was to learn and practice the experiments they were responsible for. As a result, we learnt a lot of basic Pharmacology, while the students learnt such medically-important points as why saline is better than tap-water if you want to keep tissues alive.

It was while I was setting up and testing the class experiment on the guinea-pig ileum that an event occurred which led to my first research publication, when Q came up to me with an ampoule containing a white powder and said "Ah, Brown, when you've finished would you like to try some of this?" So I made up some of the powder in Tyrode solution, added it to the bath, and the piece of intestine contracted massively and apparently irreversibly since I couldn't relax it by extensive washing. When I showed Q, he replied "I thought it might". And then revealed that the powder was a sample of the α-toxin of *Staphylococcus pyogenes* (now *S.aureus*) and introduced me to Brian Prichard, who showed me some records he had made of a similar effect of the toxin on the rabbit jejunum. So we combined forces, did some more experiments and published our joint first paper in the *British Journal of Pharmacology* **[2]**.

A little later, this paper led to an invitation by Professor Helena Raskova in Charles University, Prague, to contribute to a symposium at the 2nd International Pharmacological Congress in Prague in 1963. This prompted some more experiments with the toxin, mainly to find out to what extent its contractile effects on smooth muscle resulted from the release of endogenous spasmogens such as histamine [**3**]. Although it could release histamine, especially from guinea-pig lung, my conclusion was that its spasmogenic and vasoconstrictor effects were directly on the smooth muscles [4,**5]**. It emerged much later that the α-toxin (α-hemolysin) is a transmembrane heptameric pore-forming peptide [**6**]. The visit to the Prague congress was my first venture behind the 'iron curtain', lent dramatic impact by driving in from West Germany in an open-top UK sports car across a no-man's-land controlled by watchtower-mounted machine guns. At that time Czechoslovakia was under the control of the Stalinist Antonin Novotny. Following the Prague Spring and subsequent Soviet invasion (in 1968) Professor Raskova was dismissed from her post at Charles University but re-assumed her position after the 'velvet revolution' in 1989.

For my PhD studies Q set me the task of examining the action of a range of centrally-active drugs on ganglionic transmission, using the retraction of the nictitating membrane in the eye of an anaesthetized cat induced by preganglionic sympathetic nerve stimulation [**7**] as an index of transmission through the superior cervical sympathetic ganglion. Drugs could then be rapidly applied to the ganglion by retrograde intra-arterial injection through the external carotid artery. Don Mason taught me the dissection and all aspects of animal care as required for a Home Office licence ; he had extensive experience of both while working in industry.

I believe Q's thinking behind my project (and those he set other PhD students) was that while CNS drugs targeted the brain, central synapses were difficult to study so peripheral synapses might provide a useful 'model' to observe some of the drugs' fundamental effects. I cannot say that I discovered anything suggesting the sympathetic ganglion as an appropriate 'model' synapse. Thus, a wide range of drugs produced a uniformly-depressant action on the postganglionic responses to preganglionic nerve stimulation or to injections of acetylcholine or potassium chloride, irrespective of whether their central actions were 'depressant' (hypnotic, sedative, anticonvulsant) or 'excitatory' (alerting, convulsant, pro-epileptic), even among chemical congeners spanning the convulsant / anticonvulsant range. [**9**, **10**] Exceptionally, however, some of the barbiturates selectively antagonized the ganglion-stimulant action of acetylcholine over that of K^+ ions (and also reduced blood pressure). The probable reason for this emerged later through the work of another of Q's students, Paul Adams, who identified a channel-blocking action of pentobarbitone on frog muscle nicotinic receptors [**11**]. Nevertheless, even though the nicotinic receptor was clearly not the main target for barbiturates, on the basis of the interesting structure-activity relations among barbiturate compounds, ranging from depressant to convulsant . I concluded that the barbiturates "might interact with a specific barbiturate-sensitive transmitter system" (pp 192ff in [**12**]) that was not represented in the ganglion. I was both right and wrong on this. Later work did identify another target, the GABA receptor (e.g.[**13**]) (the transmitter function for which in the mammalian nervous system was not generally accepted in the fifties). And I was wrong because the GABA receptor *was* present in the ganglion [**14,15**] (though not involved in mainline transmission) and we found barbiturates to potentiate the action of GABA on these receptors [**16**], just like their effect on central $GABA_A$ receptors.

Having completed my thesis I received an oral examination by Professor Wilhelm Feldberg under the austere portrait of Sir Henry Dale and preceded (less austerely) by the offer of a glass of sherry and a cigar (both regretfully declined). Of the many questions asked, I remember one in particular. When asked what was the most interesting aspect of my work I started rabbiting on about structure-action relations but Feldberg abruptly stopped me and instead drew attention to some experiments I had done suggesting an anticholinesterase action of chloral hydrate [**8**], a

completely unexpected effect. He was right – the unexpected can change the direction of research (though not in this case, since it was quite trivial) and indeed might be considered a prime reason for doing experiments.

Having gained a PhD (in 1961) I was appointed to my first faculty job at Barts, that of Assistant Lecturer in Pharmacology (a non-tenured 4-year position). This presented an opportunity to try some further experiments on sympathetic ganglia. One set of experiments was prompted by a visit to London of George Koelle from the University of Pennsylvania, who expounded a new hypothesis of a 'cholinergic link' in transmitter release from sympathetic preganglionic fibres – namely, that the initial role of acetylcholine released by the afferent nerve impulse was to trigger the release of additional acetylcholine by a positive feedback effect and that it was this additional release that mediated transmission [**17**] Koelle's idea was prompted by his observation that, in sympathetic ganglia, histochemically-stained acetylcholine esterase was predominantly associated with the preganglionic terminals, instead of the postjunctional cell as at the neuromuscular junction, and so might serve to prevent excessive acetylcholine-induced transmitter release. Experimentally, it was supported by the occurrence of spontaneous postganglionic activity following anticholinesterase drug treatment and by the absence of 'denervation supersensitivity' to acetylcholine after preganglionic nerve degeneration (unlike skeletal muscle); indeed the denervated ganglion was strongly *subsensitive* to the cholinesterase-resistant agonist, carbachol.

I tried to test Koelle's hypothesis further in two ways: by recording the effects of preganglionic nerve degeneration on the action of acetylcholine more directly *in vitro* as a postsynaptic depolarization [**18**] and by testing whether acetylcholine could actually release radiolabelled acetylcholine from perfused sympathetic ganglia [**19**]. Neither supported Koelle's idea. While, the denervation tests confirmed the absence of 'denervation supersensitivity' to acetylcholine, there was no subsensitivity to carbachol; and neither acetylcholine itself nor other nicotinic receptor stimulants appeared capable of releasing radiolabelled acetylcholine.

Another topic of increasing interest in the sixties concerned the presence of muscarinic acetylcholine receptors in autonomic ganglia, one source of uncertainty being the small and variable nature of the measured responses. The *in vitro* extracellular method we had developed to record ganglion depolarization provided clarity in showing a clear hyoscine (scopolamine)-sensitive depolarization by muscarine and a partial hyoscine-sensitivity (plus, hexamethonium-insensitivity) of the responses to acetylcholine and other choline esters, but not to nicotine [**20**]. Much later we discovered that the muscarinic depolarization of ganglion cells resulted from the activation of the pirenzepine-sensitive M_1 mAChR subtype [**21**] – the first demonstration of functional M_1 receptors in the nervous system.

A sojourn in Chicago.

While at Barts I attended a lecture by Peter Waser from Zurich University [**22**] in which he showed some autoradiographs of ^{14}C-tubocurarine neatly lined up over the end-plate region of mouse diaphragm muscles. This seemed a promising way of looking at acetylcholine receptors so long as a method for immobilizing reversibly-bound radioligands could be found (bungarotoxin had not been discovered in 1960). So in 1965 I took an opportunity to visit the University of Chicago where Lloyd Roth and Walter Stumpf had developed a method for high-resolution autoradiography of water-soluble compounds. For this, radiolabelled tissues were quick frozen in liquid isopentane at -187^{o}C, cut at ≤-60^{o}C into ~1 µm sections , the sections freeze-dried then dry-mounted on emulsion-coated slides and exposed at -15^{o}C. To test the method I looked at the distribution of some freely-mobile extracellular space indicators (^{3}H-methoxyinulin, ^{3}H-D-mannitol and sodium^{35}S-sulphate) in cat sympathetic ganglia after close arterial injection [**23**]. Around 85-90% of silver grains were retained in interstitial spaces outside neurons with no more than 10-15% of the interstitial over the neurons; most of the latter could be attributed to a small amount of neuronal uptake, the actual resolving power of the method for ^{3}H beta-emission from a fixed source being <1 µm.

Armed with this information I then examined the distribution of similarly-injected ^{3}H-nicotine in the cat ganglion [**24**]. In contrast to inulin, there was a higher ^{3}H-nicotine grain density over the neuron perikarya than over the surrounding tissue. Though clearly not due to receptor-binding itself, two observations suggested this to be partly consequential on nicotinic receptor activation: the concentration of nicotine in the sympathetic neurons exceeded that in the neurons of the adjacent nicotine-insensitive vagal nodose ganglion; and uptake into the sympathetic neurons could be reduced by hexamethonium. On returning to Barts, we confirmed this receptor-dependent uptake of nicotine into isolated rat sympathetic ganglia [**25**] and found out that this could be attributed to 'base-trapping' of protonated nicotine induced by a fall in intracellular pH [**2**6] following Na^{+} entry and metabolic stimulation consequent upon nicotinic depolarization [**27,28]** with subsequent electrogenic Na^{+} extrusion by the Na^{+}/K^{+}-ATPase[**29**].

Adventures with GABA

In the Spring semester of 1970 I paid a return visit to the University of Chicago, ostensibly to help teach the new neurobiology course. By then GABA (γ-aminobutyric acid) was being taken seriously as a candidate mammalian neurotransmitter, and Iversen & Neal [**30**] had described an uptake system for ^{3}H-GABA into rat cortical brain slices, potentially into the neurons and/or nerve terminals from which GABA might be released. If so, this might provide an opportunity to use the Chicago autoradiographic method to identify 'GABAergic' neurons. With this in mind, I thought to

test the specificity of the uptake system for GABAergic neurons by comparing ^{3}H-GABA uptake into rat brain slices with that into sympathetic ganglia which (I assumed) did not contain GABAergic neurons. Surprisingly, however, the ganglia accumulated as much radioactivity on a dpm/mg basis as the brain tissue, although they took rather longer to do so. Hence I deferred the autoradiography *pro tem* pending resolution of this conundrum (and in the event was 'scooped' later that year by Tomas Hokfelt [**31**]).

An opportunity to extend this work occurred back at Barts in September of that year through the advent of Norman Bowery as a PhD student, when I suggested he might like to try to find out more about the ganglionic uptake system. Within a few months he had accumulated enough data to present a communication to the Physiological Society [**32**] and published his principal observations a year later [**33**]. In essence he confirmed a concentrative uptake of ^{2}H-GABA in sympathetic ganglia and revealed a similar uptake in some other peripheral nerves but not into any non-neural tissues. He noted that ganglionic uptake was similar to that in cortical tissue except for its slower time course and small but crucial differences in substrate specificity. He also showed that accumulated ^{2}H-GABA could be released by depolarizing the cells of origin with K^{+} but not by selectively depolarizing the neurons with carbachol. This led to the suggestion that the source of GABA release (and hence by inference, the site of uptake) were the neuroglial cells, not the neurons; this we later confirmed by autoradiography [**34,35**] (see **Fig.1**).

[**Fig.1.near here**]

Norman went on to complete a comprehensive analysis of this 'extrasynaptic' peripheral GABA system in the sympathetic ganglion. I have described this in some detail in [**36**]; his main findings may be summarized as follows. (1). He and his colleagues discovered that accumulated GABA was metabolized to succinate by the GABA – α-keto acid transaminase enzyme and then to citrate by succinic semialdehyde dehydrogenase [**37**]. This implied that glial uptake probably exerted a protective effect [**38**] by maintaining a low interstitial GABA concentration (≤1 μM [**35**]). (2). Following a report by Chet DeGroat [**39]** Norman described a depolarizing action of GABA on mammalian sympathetic ganglia *in vitro* and showed that the ligand-selectivity accorded with that of $GABA_A$ receptors in the mammalian central nervous system [**40**]. But why depolarizing, not hyperpolarizing? Paul Adams and I solved this using intracellular microelectrode recording: GABA increased ganglion cell chloride conductance as in central neurons, but this caused depolarization because the ganglion cells contained a high intracellular chloride concentration (estimated at around 24 mM, giving E_{GABA} = -42mV) [**45**]. A comparable depolarizing response to GABA has been recorded by others in peripheral sensory neurons (see [**36]**) and in peripheral unmyelinated fibers [**41**]. Later work (see [**36**]) suggests that this depolarization reflects the low expression of the outward KCC2 K^{+}/Cl^{-} transporter in peripheral neurons. (3) Noting the low threshold for

sympathetic neuronal depolarization by GABA (≤1 µM [**40**]) we asked the question whether GABA might be released from glial cells in sufficient quantity to depolarize the adjacent neurons. Norman tested this by applying substrates for the glial carrier which, of themselves, had negligible effect on the neuronal receptors but accelerated the efflux of ^{3}H-GABA by exchange-diffusion. These produced a clear depolarization of the neurons. This was ascribed to the glial release of GABA because it was reduced when the glial carrier was inhibited by omitting Na^{+} ions and increased when the intraglial concentration of GABA was increased by a preloading method [**43**]. Many years later, this form of glial-to-neuronal GABA signalling was detected in the central nervous system (see [**36**])

From ganglion to brain *in vitro*

Another connection with the University of Chicago (UC) came in the late sixties with a visit to Barts from Professor John Harvey from UC, then spending a sabbatical with Henry McIlwain at the Institute of Psychiatry in London. John was struck by the method of recording evoked electrical activity from isolated guinea-pig olfactory cortex slices used in McIlwain's department by Dr. C. Yamamoto [**44**] and wondered whether we might try this at Barts. Yamamoto's interface bath seemed a bit complicated to us and ill-suited for drug application so we said "why not treat the slice as a ganglion?". So we switched to partly-submerged slices and lower temperatures to improve oxygen supply and reduce oxygen demand. This allowed thicker slices (nominally 0.5 mm), which in turn enabled us to preserve the functional integrity of the circuit necessary to record the polysynaptic inhibitory evoked responses (surface-positive wave) following lateral olfactory stimulation, as well as the previously-recorded monosynaptic excitatory potentials. [**45**]

Following this, Norman Scholfield in our lab went on to obtain some impressively stable intracellular recordings from pyramidal neurons in these olfactory cortex slices, lasting up to 16 hrs [**46**]. He also recorded a prolonged GABA-mediated depolarizing recurrent inhibitory postsynaptic potential after lateral olfactory stimulation [**47**] and showed that this was dramatically intensified and prolonged by pentobarbitone [**48**] in keeping with the postulated $GABA_AR$ target for barbiturates [**13**, **16,**]. Martin Galvan, an enterprising PhD student, also showed that extracellular recording from the isolated olfactory cortex preparation could be used to quantitate the actions of GABA and its analogs on central neurons [**49, 50**] and the effects of intracellular uptake and release on the action of GABA [**51,52]**) just as Norman Bowery had done with sympathetic ganglia. Apart from their intrinsic interest, these various experiments helped to demystify the use of *in vitro* brain slices in pharmacology and physiology. The invention of the single microelectrode voltage-clamp technique [**53]** provided a further advance in brain slice recording; we were among the earliest pioneers applying this technique to mammalian central neurons (e.g., [**54-56**])

Galveston and M-current

In 1979 I paid the first of three visits to the Department of Biophysics at the University of Texas Medical Branch (UTMB) in Galveston, Texas, to renew (see [**41**]) a collaboration with Paul Adams. The ostensible aim of the visit was to test a hypothesis for our previously-observed potentiation of GABA responses by pentobarbitone [**16**], drawing on Paul's observations on the interaction of pentobarbitone with nicotinic receptors [**11**]. Unfortunately, however, in our hands the neuron of choice for two-electrode voltage clamp – bullfrog lumbar sympathetic neurons – gave rather poor and inconsistent responses to GABA. So instead we decided to investigate the effect of stimulating muscarinic acetylcholine receptors, since ongoing work in London [**57**] suggested some interesting properties of this response. Not only was the depolarization accompanied by an apparent reduction in input resistance suggestive of a fall in K^+ conductance as previously suggested [**58**]) but it was also accompanied by a striking facilitation, or sometimes induction, of repetitive action potential firing (**Fig.2A**).

[Fig 2 hear here]

We found that we could replicate these effects in bullfrog sympathetic neurons, and that, under voltage-clamp, muscarine produced an inward (depolarizing) current accompanied by a fall in membrane conductance as indicated by the reduced amplitude of the currents produced by hyperpolarizing voltage commands (**Fig 2B**) The most interesting event, however, was the change in the character of the current response to the voltage step. Under control conditions (before adding muscarine), the current showed a slow inward relaxation, accompanied by a decline in membrane conductance (Fig.2B legend). We reasoned that this signalled the slow closure of some ion channels (identified by other experiments as K^+-channels) that were already open at the initial holding potential. The effect of muscarine was to close these channels, eliminating these relaxations, and generating a steady inward (depolarizing) current at the holding potential. Its sensitivity to muscarine led us to dub the current an 'M-current' (I_M). In effect, the M-current acts as a braking current on neuronal excitability, and muscarine enhances excitability by releasing the brake (as in **Fig 2A**). The properties of the M-current, its inhibition by muscarine, and (importantly) the contribution of its inhibition to the slow cholinergic excitatory postsynaptic potential (sepsp) and current (sepsc) in bullfrog sympathetic neurons, were subsequently described in detail [**60-62**]. Initially, some of our observations were met with a degree of scepticism, primarily because the M-current was very small (<5% of the delayed rectifier current [**60**]) and not always easy to record. Notwithstanding I_M (or its suppression) can have a dramatic effect on neuronal excitability because there are often few other active membrane currents over much of the M-current range (upward from -60 mV [**63**])

Over the next few years, both in the USA and back in London, we continued to explore the distribution and regulation of the M-current. For example, we identified M-like currents in mammalian sympathetic [**64**] and (much later) sensory [**65**] neurons, mammalian central neurons such as hippocampal pyramidal neurons [**54, 66,**] and olfactory cortex pyramidal neurons [**67**], and human neocortical neurons [**68**]. We also showed that M-current inhibition contributed to the cholinergic sepsc in rat sympathetic ganglia [**69**], and in rat hippocampal slices in culture [**66**]. Around the same time, Sims et al [**70**] discovered a muscarine-sensitive M-current in toad gastric smooth muscle (a forerunner of much more recent work on M-currents in many mammalian smooth muscles [**71**]).

We also broadened the range of M-current inhibitors to include: Ba^{2+} ions [**72],** which directly close mammalian M-channels in outside-out membrane patches [**73**]; luteinizing hormone releasing hormone (LHRH, GnRH) [**74**]; substance P [**75**]), uridine triphosphate (UTP) [**61**]; and in rat sympathetic neurons, angiotensin [**64, 76**], bradykinin [**77**,], and UTP and other nucleotides [**78**] (see also [**79**].

Other travels and collaborations.

In 1984 I visited Beat Gahwiler (then working at Sandoz AG in Basel, Switzerland) for a very productive few weeks looking at his organotypic hippocampal slices, These slices, so beautifully prepared by Lotte Rietschen, were a revelation in terms of cell visualization, ease of microelectrode impalement and speed of drug application. When co-cultured with septal explants, the hippocampus was innervated by a rich cholinergic output from the septum. I had brought an Axoclamp switch-clamp amplifier with me, so we could voltage-clamp the hippocampal neurons and record the cholinergic slow epsc [**66**]. We showed that this, and the response to muscarine, were due (in part at least) to M-current inhibition . In between times, we also used the opportunity to obtain the first voltage-clamp recordings of the inwardly-rectifying current activated by stimulating $GABA_B$ receptors [**80**]. During a subsequent visit to Beat's lab (now in Zurich) we combined voltage-clamp and intracellular Ca^{2+} measurements to sort out *inter alia* the relation between the effects of muscarine and a beta-adrenoceptor agonist on the post-spike Ca^{2+} transient and the associated slow Ca^{2+}-activated K^+ current [**81**]

In 1985 I was invited to take up a Fogarty Scholar-in-Residence award at the Fogarty International Center of the National Institutes of Health (Bethesda, MD, USA) which I split into two 6-month stints in 1985 and 1986. I spent the first period partly in the Center and partly in the labs of Mike Brownstein and Julie Axelrod in the National Institutes of Mental Health (NIMH), in the latter trying out some K^+ channel flux studies [**82**] with a view to possible later use to assay K^+ channel blocking activity. Much of the second period (1986) was spent in Marshall Nirenberg's lab

in the National Heart Lung and Blood Institute (NHLBI), where I teamed up with Haruhiro Higashida studying the electrophysiological properties of some of Marshall's clonal cell lines. The NG108-15 mouse neuroblastoma x rat glioma cell looked interesting since it appeared to possess an M-like current [**83, 84**], This was rather insensitive to muscarinic agonists (wrong muscarinic receptor [**88**]) but was potently inhibited by bradykinin [**85, 87**]. Inhibition was preceded by a large outward current due to the release of intracellular Ca^{2+} and activation of a species of Ca^{2+}-dependent K^+ channel [**85,,87].** The initial response to bradykinin could be imitated by the intracellular injection of inositol-1,4,5-trisphosphate (IP_3) or Ca^{3+} whereas the later response (M-current inhibition) could be imitated by bath-application of phorbol esters, suggesting that both responses might have resulted from the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) to IP_3 and diacylglycerol (DAG) (see further below). (The experiments in [**87**] involved a real *tour-de-force* of electrode manipulation by Haruhiro Higashida with a cell-attached pipette to record K_{Ca} channels, up to 3 intracellular microelectrodes (one to clamp the membrane potential, and two to inject IP_3 or Ca^{2+}) and a focal extracellular electrode to apply bradykinin.

At NIH, Haru and I met up with Professor Shosaku Numa, another Fogarty scholar on leave from Kyoto University. At the time Numa was busy cloning the different muscarinic receptor subtypes , and the meeting at NIH led to subsequent collaborative studies using the NG108-15 cells for functional expression and differentiation of muscarinic receptor subtypes. [**88, 89**] We continued using the NG108-15 cells (and other cell lines) for various other purposes post-NIH – for example, where their large size and robustness allowed otherwise traumatic procedures, such as the microinjection of specific G protein antibodies to test receptor-ion channel transduction pathways [**90**]; or where they express novel receptors, such as the P2Y2 purinergic receptor which seemed to target both voltage-gated Ca^{2+} and M-type K^+ channel [**91,92**]. To find out whether both of these effects were mediated by the same genotypic receptor Sasha Filippov and I embarked on a sustained and fruitful collaboration with Eric Barnard, in which we used rat sympathetic neurons as expression vectors to express mRNAs or cDNAs for each of the known P2Y receptors and studied their coupling to endogenous or expressed ion channels (see, e.g., [**78**]) Thus, both of the effects described in [**91,92**] could be replicated in sympathetic neurons by expressing a single P2Y2R genotype [**ref 93**].

M-channel composition

We had hoped that NG108-15 might also provide a route to identifying M-channel genes by expression cloning but to no avail. Instead, our colleagues in Japan isolated two other members of the Kv K^+ channel family from these cells:, NGK1 (Kv1.2), and NGK2 (Kv3.1), the first mammalian member of the *Shaw* family [**94**]. With Cathy Stansfeld, we also set up a collaboration with Olaf Pongs in ZMNH, Hamburg, to express some of his K^+ channel cDNAs. None corresponded to M-

channels but one (rat *eag*; Kv10.1) showed an interesting similarity in that the current was inhibited by muscarine, through a rise in intracellular Ca^{2+} [**95**]

The M-channel composition eventually emerged from positional cloning of genetic mutations associated with human epilepsy (summarized in [**96**]). These yielded two genes: *KCNQ2* and *KCNQ3*, homologous with *KCNQ1* (encoding the cardiac K^+ channel KvLQT1). David McKinnon and colleagues then showed that mRNAs for *KCNQ2* and *KCNQ3* yielded an authentic M-current when co-injected into frog oocytes [**97**]. In our lab, we showed that all of the then-extant *KCNQ* genes (*KCNQ* 1-4) yielded M-like currents when expressed from cDNA monomers in mammalian CHO cells, and that all such currents, including that generated by co-expressed *KCNQ2+3* heteromers, were susceptible to inhibition by a muscarinic receptor agonist [**98**]; and that the single channel currents of co-expressed *KCNQ2+3* cDNAs in CHO cells [**99**] were comparable to those of native M-channels in rat sympathetic neurone [**100**]. We then used the differential sensitivities of KCNQ2 and KCNQ3 currents to tetraethylammonium (TEA) **[101]** to deduce that native M-currents were likely composed of KCNQ2 and KCNQ3 in 1:1 stoichiometric proportions, presumably as a 2+2 tetramer [**102**]. The same appeared true for much of the M-current in hippocampal neurons [**103**] We also tried some complementary experiments using selective subunit disruption to determine subunit composition in native M-channels. Thus, expression of the cDNA for a non-conducting dominant negative *KCNQ3* mutation strongly reduced the native M-current in rat sympathetic neurons and eliminated the linopirdine-sensitive component of M-like current in NG108-15 cells [**105**], the residual current NG108-15 current, carried by a *merg1a* gene product [**105**] being eliminated by a non-conducting variant of *merg1a* [**104**]. Much later, we demonstrated a crucial role for *KCNQ2* in the M-current using sympathetic neurons from embryos of transgenic mice homozygote for a truncated (non-functional) variant of *KCNQ2* in which the M-current was completely abolished [**106**]

Receptor – M-channel transduction in sympathetic ganglia.

Indirect coupling. The overlapping inhibition of M-current in the same cell by several different extracellular messengers via different receptors suggested that they probably acted indirectly on the channels using one or more intracellular biochemical transducers in common [**63**]. This indirect action was substantiated when Sasha Selyanko found that the activity of single M-channels in rat sympathetic recorded within cell-attached patch pipettes could be readily inhibited by muscarine applied to the cell membrane *outside* the patch, but not by adding muscarine to the pipette solution [**107**] (see **Fig.3**) (see also [**108**] for frog neuron channels). This implies the movement of a messenger generated by receptors outside the patch to channels inside the patch (or, as later revealed, in the reverse direction).

[Fig. 3 near here]

The phosphoinositide pathway. One plausible signalling pathway activated by M1 muscarinic receptors [**109**] is hydrolysis of membrane phosphatidylinositol-4,5-bisphosphate (PIP_2) with the production of two mobile messengers, inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) (later reviewed in [**110**]) IP_3 may release Ca^{2+} ions from the endoplasmic reticulum, to act as a tertiary messenger; DAG may activate protein kinase C (PKC). Martin Larrabee showed the acetylcholine-induced incorporation of phosphate into phosphatidylinositol in sympathetic ganglia in 1965 [**111**], and concluded a lecture in London that year with the words *"Phosphatidyl? 'e knows it all!"* Much later, we confirmed the muscarinic hydrolysis of PIP_2 in this tissue biochemically [**112]** and optically **[113]**

G proteins*.* The first step following receptor stimulation is activation of a G protein. We characterized the functional G protein linking M1 muscarinic receptors to M-current inhibition in sympathetic neurons as Gq (and/or G11) by: (a) injecting selective G protein $\alpha_{q/11}$-subunit blocking antibodies into sympathetic neurones [**114**]; (b) expressing selective antisense $G\alpha_q$ oligonucleotides from microinjected cDNA constructs [**115**]' or (c) by applying a membrane-targeted lipidated blocking peptide [**116**]. By comparison, noradrenergic [**115, 116, 121**] or 'fast' (M4) cholinergic [**117**] inhibition of N-type ($Ca_V2.2$) Ca^{2+} current were not affected by these anti-Gq agents but were selectively inhibited by an anti-Go agents. Limited studies suggested that bradykinin might use a different G protein (G_{11} [**118**]) or phospholipase C isoform (PLCβ4 [**119**]) to close M-channels in sympathetic neurons.

***Ca^{2+} ions*.** Alex Selyanko found Ca^{2+} ions to close M-channels in excised inside-out membrane patches from rat sympathetic neurons with an IC_{60} of ~100nM [**120**] (later shown to result from an interaction with channel-attached calmodulin [**121**]). Functionally, Ca^{2+} release from the endoplasmic reticulum has been found responsible for M-current inhibition by bradykinin [**122**] but not by muscarine [**112**] which releases too little Ca^{2+} [**123**], though coupled to an apparently-similar amount of phosphoinositide hydrolysis [**113**, **124**]. This led us to suggest separate M-current signalling 'microdomains' for bradykinin and muscarine [**123**]. These particular receptor microdomains have been confirmed for other aspects of M-current signalling [**125**]

Diacylglycerol and protein kinase C. Both muscarinic agonists and bradykinin rapidly generate free diacylglycerol in sympathetic neurons as judged from the activation of expressed TrpC6 channels [**125**] Naoto Hoshi from Kanazawa University showed that this can induce PKC-mediated phosphorylation of the KCNQ2 M-channel subunit, facilitated by the scaffold protein AKAP *A-kinase anchoring protein[**126**]. Studying sympathetic neurons in our lab, Naoto used selective inhibitors of PKC activation by DAG to show that PKC activation by muscarinic agonists sensitized the native neuronal M-channels to muscarinic closure [**126**]. He later showed that this was due to a

reduced affinity of the M–channel to the endogenous activator PIP_2 [**127**] (see below), as we had suggested [**110**].

PIP2 as the messenger. In 1998 Don Hilgemann turned the phosphoinositide story on its head by showing that Kir channels actually needed PIP_2 for their activation and were closed by a PIP_2 antibody [**128**]. Evidence for a similar role for PIP_2 in regulating M-channel activity emerged from the labs of Bertil Hille [**129**] and Diomedes Logothetis [**130**]: Suh & Hille [**129**] showed that PIP_2 synthesis was necessary for recovery of KCNQ channels or native M-currents after muscarinic inhibition; Zhang et al [**130**] showed directly that PIP_2 could activate expressed KCNQ channels in excised oocyte membrane patches, and that this was reversed by PIP_2 antibodies. In 2005. we [**113**] specifically addressed the question whether it was the loss of membrane PIP_2 following muscarinic PIP_2 hydrolysis, rather than the products of PIP_2 hydrolysis, that was responsible for muscarinic inhibition of sympathetic neuron M-currents, by over-expressing the PIP_2 synthesizing enzyme phosphatidylinositol-4-phosphate-5-kinase (PI-5K) to increase the amount of PIP_2. Simon Hughes used a competition assay between intracellularly-dialyzed IP_3 and a PIP_2 binding probe to measure changes in membrane PIP_2. PI-5K over-expression increased membrane PIP_2 ~2.7 times but *reduced* the amount of M-current block by oxotremorine-M from 74% to <10%, instead of increasing the block (as might be expected were inhibition due to products of PIP_2 hydrolysis). Bertil Hille and colleagues have since constructed a detailed model for muscarinic inhibition of M-channels based on the PIP_2 loss mechanism [**131**] which translates well to sympathetic neurons [**132**]

In a subsequent study in our lab [**133**] Seva Telezhkin explored the kinetics of PIP_2 – M-channel gating by recording the activity of expressed heteromeric KCNQ2/KCNQ3 channels induced by rapid application of varying concentrations of a PIP_2 homolog to the inside face of isolated channels. Previous work from Mark Shapiro's lab had shown that the two subunits expressed as monomers had different sensitivities to PIP_2 [**134**]. Seva found that distinct components of PIP_2 activation attributable to PIP_2 binding to each channel subunit in the tetramer could be identified and that maximal channel opening occurs when all four subunits have bound a molecule of PIP_2.

Other discoveries by lab colleagues

I have been lucky to have many innovative and talented lab colleagues. In addition to their contributions above, they have made a variety of other individual discoveries. People include: Trevor Smart, with Khaled Houamed: oocyte expression of ion channel mRNAs [**135**]; Reg Docherty, with James Halliwell: a presynaptic nicotinic receptor [**136**]; Steve Marsh: calcium neurotoxicity via capsaicin receptors [**137**] and a PKC-activated chloride channel [**138**]; Sasha Filippov: functional $GABA_B$ receptors need two subunits [**139**]; Alex Selyanko with David MacKinnon: effect of alternative splicing on KCNQ2 function [**140**]; Jose Fernandez: functionally effective G protein expression in neurons [**141**]; Lucine Tatulian: retigabine alters KCNQ voltage-sensitivity [**142**]; Patrick Delmas: functional expression of polycystin genes in mammalian cells [**143**]; Nicolas Wanaverbecq: calcium regulation mechanisms in sympathetic neurons [**144**]; Tim Allen: acetylcholine and glutamate co-release from single basal forebrain axons [**145**]; Mala Shah: axonal M-channels controlling action potential threshold [**146**]; Gayle Passmore: M-channels in sensory nerve endings. [**147**]

Some reflections

***Career options*.** When I went to UCL (in 1954) less than 5% of the UK school population went on to higher education, compared with some 40% now. Hence going to University was itself relatively rare and *ipso facto* a common entrée to a research life. Thus, of the 13 students completing the Physiology degree at UCL in 1957, at least three others went on to fulfil a distinguished research career (Humphrey Rang, my predecessor as Chairman of Pharmacology and Director of the Sandoz/Novartis Institute at UCL; Tom Connors, Director of the MRC Toxicology Unit; and Christine Armett, Professor of Biology at U Mass, Boston, USA), while Alan Boura (a fellow student for the first two years) became Research Director at Reckitts UK, then Professor of Pharmacology at Monash University, in Australia. So, in this context my own career was nothing unusual. For each substantive step post PhD – assistant lecturer then lecturer at Barts, senior lecturer (leading to Wellcome Professor and Chairman of Pharmacology) at the University of London School of Pharmacy (SoP), a Fogarty Scholarship at NIH, and finally Chairman of Pharmacology at UCL - were initially by invitation. My decisions were then determined by the distinction of my prospective colleagues, the availability of suitable facilities and funds to pursue some interesting research, and the suitability of the location for family life. For example, at the SoP, Donald Straughan had energetically created a Department specializing in CNS electrophysiology and pharmacology, so was well-equipped for the sort of work I was attempting; while UCL already had renowned expertise in receptors and ion channels through the likes of Don Jenkinson, David Colquhoun and Stuart Cull-

Candy, with correspondingly appropriate facilities. My initial postdoc appointment as Instructor at the University of Chicago followed a specific enquiry by me about their high-resolution autoradiographic method. Shorter-term lab visits to (e.g.) Iowa City, Bethesda, Texas, Basel, Zurich and Kanazawa were by mutual arrangement with my hosts and friends John Harvey, Mike Brownstein, David Carpenter, Paul Adams, Beat Gahwiler and Haruhiro Higashida; all these visits were great fun and highly productive. Given the very different circumstances faced by the current student or postdoc I feel chary about offering career advice except to say enjoy your research – if you don't get some enjoyment out of it – even some fun – you're in the wrong lab or the wrong job.

***Funding*.** I was fortunate in that, when I started research, Universities received some basic funding for research under the "dual support" system. My research was relatively cheap: much of the apparatus required was made on-site with the help of departmental electronic and workshop engineers, and the most expensive item, animals, were bred in-house and relatively cheap at that time. My first attempt at submitting a research grant in the UK (to the Medical Research Council, MRC) was only partially successful in yielding one-year's minimal support for a "pilot study"; fortunately, that was successful enough for an upgrade to a full 3 years project grant. My ability to generate an independent research laboratory was more effectively facilitated by a grant from the American Medical Association Education and Research Foundation (AMAERF) that I applied for whilst at the University of Chicago; in addition to funding equipment and running costs, this allowed me take on a research assistant and a technician, so forming our first research "team". Subsequently, I have been very fortunate in receiving a succession of competitively-renewable 5-year Programme Grants from the UK MRC and from the Wellcome Trust. These started when I joined the School of Pharmacy (SoP), and Donald Straughan and I were awarded a joint Programme Grant from the MRC. When Donald left SoP, I re-applied for the grant and was awarded it as solo PI. This was later supplemented by an MRC "Group" grant, which provided infrastructural support such as salary for an electronic engineer. Shortly after I joined UCL, David Colquhoun and I submitted a proposal to the Wellcome Trust to create and fund a new Laboratory for Molecular Pharmacology (LMP), to complement our electrophysiological studies on ion channels and receptors with molecular biological and biochemical input. With associated Programme Grants, this ran successfully for 20 years. The 5-year grants that I have received have been beneficial in allowing flexibility in starting new lines of research and in sustaining staff appointments (e.g., being able to maintain workshop and electronic engineer positions) while avoiding the bureaucratic and financial overheads involved in running a Research Unit or Institute. Since these stemmed originally from 3-year investigator-led project grants superimposed on basic University research funding, it is doubly unfortunate that both these forms of funding have diminished over recent years in the UK; the junior faculty most affected have my sympathy.

Changes in laboratory life over sixty years. I sometimes think of what was *not* available when I started research in 1957 but are in routine use now. For example, although some institutes had mainframe computers addressed by punched cards, there were no lab PCs or cellphones, and no internet, so no online PubMed or Google search; and without photocopiers: a literature search meant going to the library, looking up indices, finding and reading the appropriate journal and making written notes. With no lab PC, experimental output was as direct hard copy: at its simplest, a scratch on a kymograph; or for electrophysiological data, photographs of an oscilloscope screen, which had to be developed in a dark room (and hoping no one comes in and turns the light on). With no word-processor and with carbon paper to make a couple of copies, writing a paper was equally laborious with little room for error (For the publishers of my early papers I made minor corrections in handwriting to avoid re-typing). Though progress was slower than now, lab life was just as busy because the simplest things took ages.

Changes in pharmacology research. From the viewpoint of research strategy, the greatest limitation compared with the present time was the complete absence of molecular biology or molecular genetics: no genes, no amino acid sequences, structures or antibodies for any ion channels or receptors (or at least any that I was trying to study). No mRNA or cDNA expression, no distribution studies using defined antibodies or mRNA hybridization. Everything about a receptor had to be deduced from functional studies using different agonists and antagonists. Given these constraints, when receptors and channels were first cloned and expressed in the mid-80s, it was encouraging how accurate the previous deductions of functional pharmacologists and physiologists like Dale, Katz, Hodgkin, Ahlquist and Black turned out to be. Now, in seeking novel targets one can start from the gene or gene products (witness the orexins and their receptors). Perhaps a start from the homologous *KNQ1* gene (had that been available in 1979) might have proved a faster way of revealing the KCNQ2/3-based M-channel.

Reflections 1133 words

Text 7475 words + 3 Figs

Original 6334 + 1 Fig

References

1. Brownlee G, Quilliam JP (1953). *Practical Pharmacology*. (University of London).

2. Brown DA, Prichard BNC, Quilliam JP (1959). Some pharmacological properties of the alpha-toxin of staphylococcus pyogenes. *Br J Pharmacol Chemother.* 14:59-67.

3. Feldberg W, Kellaway CH.(1937). The liberation of histamine by staphylococcal toxin and mercuric chloride. *Aust. J. Exp. Biol. Med. Sci*., 16, 249-259.

4. Brown DA. (1965) The release of histamine by the alpha-toxin of Staphylococcus pyogenes. *Br J Pharmacol Chemother.* 25:771-780

5. Brown DA, Quilliam JP. (1965) Some effects of staphylococcal alpha-toxin on isolated mammalian smooth muscle preparations. *Br J Pharmacol Chemother.* 25:781-789.

6. Gouaux E. (1998). alpha-Hemolysin from Staphylococcus aureus: an archetype of beta-barrel, channel-forming toxins. *J Struct Biol*. 121:110-122.

7. Feldberg W, Vartiainen (1934) Further observations on the physiology and pharmacology of a sympathetic ganglion. *J Physiol*. 83:103-128.

8. Brown DA. (1962) An eserine-like action of chloral hydrate. *Br J Pharmacol Chemother.* 19:111-119

9. Brown DA, Quilliam JP (1964) The effects of some centrally-acting drugs on ganglionic transmission in the cat. *Br J Pharmacol Chemother*. 23:241-256.

10. Brown DA, Quilliam JP (1964) Observations of the mode of action of some central depressant drugs on transmission through the cat superior cervical ganglion. *Br J Pharmacol Chemother*. 23:257-272.

11. Adams PR (1976). Drug blockade of open end-plate channels. *J Physiol*. 260:531-552.

12. Brown, D.A. (1961). “The effect of some central depressant drugs on synaptic transmission”. PhD Thesis, University of London.

13. Nicoll RA (1975) Presynaptic action of barbiturates in the frog spinal cord. *Proc Natl Acad Sci U S A.* 72:1460-1463.

14. De Groat WC. (1970). The actions of gamma-aminobutyric acid and related amino acids on mammalian autonomic ganglia. *J Pharmacol Exp Ther*. 172:384-396.

15. Bowery NG, Brown DA. (1974) Depolarizing actions of gamma-aminobutyric acid and related compounds on rat superior cervical ganglia in vitro. *Br J Pharmacol*. 50:205-218.

16. Brown DA, Constanti A. (1978). Interaction of pentobarbitone and gamma-aminobutyric acid on mammalian sympathetic ganglion cells. *Br J Pharmacol*. 63, 217-224.

17. Koelle GB. (1962) A new general concept of the neurohumoral functions of acetylcholine and acetylcholinesterase. *J.Pharm.Pharmacol.,* 14: 65-90

18. Brown DA (1966) Depolarization of normal and preganglionically denervated superior cervical ganglia by stimulant drugs. *Br J Pharmacol Chemother.* 26:511-520.

19. Brown DA, Jones KB, Halliwell JV, Quilliam JP (1970). Evidence against a presynaptic action of acetylcholine during ganglionic transmission. *Nature*. 226: 958-959.

20. Brown DA (1966) Effects of hexamethonium and hyoscine on the drug-induced depolarization of isolated superior cervical ganglia. *Br J Pharmacol Chemother*. 26: 521–537.

21. Brown, DA, Forward A, Marsh S. (1980) Antagonist discrimination between ganglionic and ileal muscarinic receptors. *Br J Pharmacol*. 1980; 71: 362–364.

22. Waser PG (1960). The cholinergic receptor. *J. Pharm, Pharmacol* 12:577-594.

23. Brown DA, Stumpf WE, Roth LJ. (1969) Location of radioactively labelled extracellular fluid indicators in nervous tissue by autoradiography. *J Cell Sci.* 4:265-288.

24. Brown DA, Hoffmann PC, Roth LJ. (1969). 3H-Nicotine in cat superior cervical and nodose ganglia after close-arterial injection in vivo. *Br J Pharmacol*. 35:406-317.

25. Brown DA, Halliwell JV, Scholfield CN. (1971). Uptake of nicotine and extracellular space markers by isolated rat ganglia in relation to receptor activation. *Br J Pharmacol*. 42, 100-113.

26. Brown DA, Halliwell JV (1972). Intracellular pH in rat isolated superior cervical ganglia in relation to nicotine-depolarization and nicotine-uptake. *Br J Pharmacol*. 45, 349-359

27. Brown DA, Scholfield CN. (1974). Changes of intracellular sodium and potassium ion concentrations in isolated rat superior cervical ganglia induced by depolarizing agents. *J Physiol*. 242, 307-319

28. Brown DA, Scholfield CN (1974). Movements of labelled sodium ions in isolated rat superior cervical ganglia. *J Physiol*. 242, 321-351

29. Brown DA, Brownstein MJ, Scholfield CN. (1972). Origin of the after-hyperpolarization that follows removal of depolarizing agents from the isolated superior cervical ganglion of the rat. *Br J Pharmacol*. 44, 651-671.

30. Iversen, LL, Neal MJ. (1968). The uptake of 3H-GABA by slices of rat cerebral cortex. *J.Neurochem*. 15: 1141-1149

31. Hökfelt T, Ljungdahl A. (1970). Cellular localization of labeled gamma-aminobutyric acid (3H GABA) in rat cerebellar cortex: an autoradiographic study. *Brain Res*. 22:391-396

32. Bowery NG, Brown DA. (1971). Observations on (3 H) γ-aminobutyric acid accumulation and efflux in isolated sympathetic ganglia. *J Physiol*. 218 Suppl:32P-33P.

33. Bowery NG, Brown DA. (1972), γ-Aminobutyric acid uptake by sympathetic ganglia. *Nat New Biol.* 238:89-91.

34. Young JA, Brown DA, Kelly JS, Schon F. (1973) Autoradiographic localization of sites of (3H)gamma-aminobutyric acid accumulation in peripheral autonomic ganglia. *Brain Res*. 63:479-486

35. Bowery NG, Brown DA, White RD, Yamini G.(1979a). [3H]gamma-Aminobutyric acid uptake into neuroglial cells of rat superior cervical sympathetic ganglia. *J Physiol*. 293:51-74

36. Brown DA (2018). Norman Bowery's discoveries about extrasynaptic and asynaptic GABA systems and their significance. *Neuropharmacology*. 136:3-9.

37. Walsh JM, Bowery NG, Brown DA, Clark JB. (1974). Metabolism of gamma-aminobutyric acid (GABA) by peripheral nervous tissue. *J Neurochem*. 22:1145-1147.

38. Brown DA, Galvan M (1977). Influence of neuroglial transport on the action of gamma-aminobutyric acid on mammalian ganglion cells. *Br J Pharmacol*. 59:373-378

39. De Groat WC. (1970). The actions of gamma-aminobutyric acid and related amino acids on mammalian autonomic ganglia. *J Pharmacol Exp Ther*. 172:384-396.

40. Bowery NG, Brown DA. (1974) Depolarizing actions of gamma-aminobutyric acid and related compounds on rat superior cervical ganglia in vitro. *Br J Pharmacol*. 50:205-218.

41. Adams PR, Brown DA. (1975) Actions of gamma-aminobutyric acid on sympathetic ganglion cells. *J Physiol.* 250:85-120.

42. Brown DA, Marsh S. (1978). Axonal GABA receptors in mammalian peripheral nerve trunks. *Brain Res* 156: 187-191

43. Bowery NG, Brown DA, Collins GG, Galvan M, Marsh S, Yamini G. (1976a). Indirect effects of amino-acids on sympathetic ganglion cells mediated through the release of gamma-aminobutyric acid from glial cells. *Br J Pharmacol*. 57:73-91.

44. Yamamoto C, McIlwain H. (1966). Electrical activities from thin sections of the mammalian brain maintained in chemically-defined media *in vitro. J Neurochem.*13:1333-1342.

45. Harvey JA, Scholfield CN, Brown DA. (1974) Evoked surface-positive potentials in isolated mammalian olfactory cortex. *Brain Res*. 76:235-245.

46. Scholfield CN.(1978) Electrical properties of neurones in the olfactory cortex slice *in vitro*. *J Physiol*. 275:535-546

47. Scholfield CN (1978) A depolarizing inhibitory potential in neurones of the olfactory cortex *in vitro*. *J Physiol*. 275:547-557

48. Scholfield CN (1978c).A barbiturate induced intensification of the inhibitory potential in slices of guinea-pig olfactory cortex.*J Physiol*. 275:559-566

49. Brown DA, Galvan M. (1979) Responses of the guinea-pig isolated olfactory cortex slice to gamma-aminobutyric acid recorded with extracellular electrodes.*Br J Pharmacol*. 65:347-353

50. Brown DA, Scholfield CN.(1979). Depolarization of neurones in the isolated olfactory cortex of the guinea-pig by gamma-aminobutyric acid.*Br J Pharmacol*. 65:339-345.

51. Brown DA, Collins GG, Galvan M. (1980). Influence of cellular transport on the interaction of amino acids with gamma-aminobutyric acid (GABA)-receptors in the isolated olfactory cortex of the guinea-pig. *Br J Pharmacol*. 68:251-262.

52. Brown DA, Scholfield CN. (1984) Inhibition of GABA uptake potentiates the conductance increase produced by GABA-mimetic compounds on single neurones in isolated olfactory cortex slices of the guinea-pig.*Br J Pharmacol*. 83:195-202.

53. Wilson WA, Goldner MM. (1975). Voltage clamping with a single microelectrode. *J.Neurobiol*. 6: 411-422.

54. Halliwell J V, Adams, PR. (1982). Voltage clamp analysis of muscarinic excitation in hippocampal neurones. *Brain Res*. 250, 71-92

55. Constanti A, Galvan M (1983) Fast inward-rectifying current accounts for anomalous rectification in olfactory cortex neurones. *J Physiol*. 335:153-178

56. Brown DA, Griffith WH.(1983) Calcium-activated outward current in voltage-clamped hippocampal neurones of the guinea-pig. *J Physiol*. 337:287-301

57. Brown DA, Constanti A. (1980) Intracellular observations on the effects of muscarinic agonists on rat sympathetic neurons. *Br J Pharmacol* 70:593-608.

58. Weight FF, Votava Z (1970). Slow synaptic excitation in sympathetic ganglion cells: evidence for synaptic inactivation of potassium conductance. *Science* 170: 735-738.

59. Brown DA, Adams PR. (1980) Muscarinic suppression of a novel voltage-sensitive K+ current in a vertebrate neurone. *Nature*. 283:673-676

60. Adams PR, Brown DA, Constanti A. (1982) M-currents and other potassium currents in bullfrog sympathetic neurones. *J Physiol* 330: 537-572.

61. Adams PR, Brown DA, Constanti A. (1982) Pharmacological inhibition of the M-current. *J Physiol*. 332: 223-262.

62. . Adams PR, Brown DA. (1982) Synaptic inhibition of the M-current: slow excitatory postsynaptic mechanism in bullfrog sympathetic neurones. *J Physiol* 332: 263-272, 1982.

63. . Brown DA. (1988). M currents. In *Ion Channels*, vol 1, pp 55-99, ed. T.Narahashi (New York: Plenum Press).

64. Constanti A, Brown DA. (1981) M-currents in voltage-clamped mammalian sympathetic neurones. *Neurosci Lett* 24: 289-294.

65. Passmore GM, Selyanko AA, Mistry M, Al-Qatari M, Marsh SJ, Matthews EA, Dickenson AH, Brown TA, Burbidge SA, Main M, Brown DA. (2003) KCNQ/M currents in sensory neurons: significance for pain therapy. *J Neurosci.* 23: 7227-7236

66. Gahwiler BH, Brown DA. (1985) Functional innervation of cultured hippocampal neurones by cholinergic afferents from co-cultured septal explants. *Nature*. 313: 577-579.

67. Constanti A, Sim JA.(1987) Muscarinic receptors mediating suppression of the M-current in guinea-pig olfactory cortex neurones may be of the M2-subtype. *Br J Pharmacol.* 90: 3-5.

68 Halliwell JV (1986) M-current in human neocortical neurones. *Neurosci Lett*. 67:1-6.

69 Brown DA, Selyanko AA. (1985) Membrane currents underlying the slow excitatory post-synaptic potential in the rat sympathetic ganglion. *J Physiol.* 365: 335-364

70 Sims SM, Singer JJ, Walsh JV Jr. (1985) Cholinergic agonists suppress a potassium current in freshly dissociated smooth muscle cells of the toad. *J Physiol*. 367: 503-529

71. Greenwood IA, Ohya S. (2009) New tricks for old dogs: KCNQ expression and role in smooth muscle. *Br J Pharmacol*. 156:1196–1203.

72. Constanti A, Adams PR, Brown DA. (1981). Why do barium ions imitate acetylcholine? *Brain Res.* 206:244-250.

73. Stansfeld CE, Marsh SJ, Gibb AJ, Brown DA. (1993).Identification of M-channels in outside-out patches excised from sympathetic ganglion cells. *Neuron* 10: 639-654.

74. Adams PR, Brown DA. (1980). Luteinizing hormone-releasing factor and muscarinic agonists act on the same voltage-sensitive K+-current in bullfrog sympathetic neurones. *Br J Pharmacol.* 68: 353-355.

75. Adams PR, Brown DA, Jones SW. (1983) Substance P inhibits the M-current in bullfrog sympathetic neurones. *Br J Pharmacol.* 79:330-333.

76.Brown DA, Constanti A, Marsh S (1980) Angiotensin mimics the action of muscarinic agonists on rat sympathetic neurones. *Brain Res*. 193:614-619

77.Jones S, Brown DA, Milligan G, Willer E, Buckley NJ, Caulfield MP. (1995) Bradykinin excites rat sympathetic neurons by inhibition of M current through a mechanism involving B2 receptors and G alpha q/11. *Neuron*. 14: 399-405.

78. Brown DA, Filippov AK, Barnard EA.(2000) Inhibition of potassium and calcium currents in neurones by molecularly-defined P2Y receptors. *J Auton Nerv Syst*. 81:31-36

79. Boehm S. (1998) Selective inhibition of M-type potassium channels in rat sympathetic neurons by uridine nucleotide preferring receptors. *Br J Pharmacol*. 124:1261-1269

80. Gähwiler BH, Brown DA.(1985) GABAB-receptor-activated K+ current in voltage-clamped CA3 pyramidal cells in hippocampal cultures. *Proc Natl Acad Sci U S A*. 82:1558-1562.

80. Gähwiler BH, Brown DA. (1985). GABAB-receptor-activated K+ current in voltage-clamped CA3 pyramidal cells in hippocampal cultures. *Proc Natl Acad Sci U S A*. 82:1558-1562

81. Knöpfel T, Vranesic I, Gähwiler BH, Brown DA (1990). Muscarinic and beta-adrenergic depression of the slow Ca2(+)-activated potassium conductance in hippocampal CA3 pyramidal cells is not mediated by a reduction of depolarization-induced cytosolic Ca2+ transients. *Proc Natl Acad Sci U S A.* 87:4083-4087.

82. Luini A, Brown DA (1990). Effects of corticotrophin releasing factor, muscarine and somatostatin on rubidium and potassium efflux from mouse AtT-20 pituitary cells. *Eur J Neurosci*. 2:126-131.

83. Higashida H, Brown DA. (1986) Two polyphosphoinositide metabolites control two K^+ currents in a neuronal cell. *Nature*, 323: 333-335.

84. Brown DA, Higashida H.(1988a) Voltage- and calcium-activated potassium currents in mouse neuroblastoma x rat glioma hybrid cells.*J Physiol*. 397:149-165

85.Brown DA, Higashida H.(1988b) Membrane current responses of NG108-15 mouse neuroblastoma x rat glioma hybrid cells to bradykinin.*J Physiol*. 397:167-184.

86. Brown DA, Higashida H.(1988c) Inositol 1,4,5-trisphosphate and diacylglycerol mimic bradykinin effects on mouse neuroblastoma x rat glioma hybrid cells. *J Physiol*. 397:185-207

87. Higashida H, Brown DA. (1988d). Ca2+-dependent K+ channels in neuroblastoma hybrid cells activated by intracellular inositol trisphosphate and extracellular bradykinin.*FEBS Lett*. 238:395-400.

88. Higashida H, Hashii M, Fukuda K, Caulfield MP, Numa S, Brown DA (1990) Selective coupling of different muscarinic acetylcholine receptors to neuronal calcium currents in DNA-transfected cells..*Proc Biol Sci.* 242:68-74

89.. Robbins J, Caulfield MP, Higashida H, Brown DA.(1991) Genotypic m3-muscarinic receptors preferentially Inhibit M-currents in DNA-transfected NG108-15 neuroblastoma x glioma hybrid cells.*Eur J Neurosci*. 3:820-824

90. McFadzean I, Mullaney I, Brown DA, Milligan G.(1989), Antibodies to the GTP binding protein, Go, antagonize noradrenaline-induced calcium current inhibition in NG108-15 hybrid cells. *Neuron*. 3:177-182

91.. Filippov AK, Selyanko AA, Robbins J, Brown DA (1994) Activation of nucleotide receptors inhibits M-type K current [IK(M)] in neuroblastoma x glioma hybrid cells. *Pflugers Arch*. 429:223-230.

92. Filippov AK, Brown DA.(1996) Activation of nucleotide receptors inhibits high-threshold calcium currents in NG108-15 neuronal hybrid cells.*Eur J Neurosci*. 8:1149-1155.

93.. Filippov AK, Webb TE, Barnard EA, Brown DA (1998) P2Y2 nucleotide receptors expressed heterologously in sympathetic neurons inhibit both N-type Ca2+ and M-type K+ currents. *J Neurosci.* 18:5170-5179

94. Yokoyama S, Imoto K, Kawamura T, Higashida H, Iwabe N, Miyata T, Numa S (1989). Potassium channels from NG108-15 neuroblastoma-glioma hybrid cells. Primary structure and functional expression from cDNAs *FEBS Lett*. 259:37-42.

95.. Stansfeld CE, Roper J, Ludwig J, Weseloh RM, Marsh SJ, Brown DA, Pongs O (1996) Elevation of intracellular calcium by muscarinic receptor activation induces a block of voltage-activated rat *ether-a-go-go* channels in a stably-transfected cell line. *Proc Natl Acad Sci USA* 93: 9910-9914.

96 Jentsch TJ. (2000) Neuronal KCNQ potassium channels: physiology and role in disease. *Nat Rev Neurosci.* 1: 21-30, 2000.

97. Wang HS, Pan Z, Shi W, Brown BS, Wymore RS, Cohen IS, Dixon JE, McKinnon D.(1998) KCNQ2 and KCNQ3 potassium channel subunits: molecular correlates of the M-channel. *Science.* 282: 1890-1893.

98. Selyanko AA, Hadley JK, Wood IC, Abogadie FC, Jentsch TJ, Brown DA. (2000) Inhibition of KCNQ1-4 potassium channels expressed in mammalian cells via M1 muscarinic acetylcholine receptors. *J Physiol*. 522: 349-355.

99. Selyanko AA, Hadley JK, Brown DA. (2001) Properties of single M-type KCNQ2/KCNQ3 potassium channels expressed in mammalian cells. *J. Physiol* 534: 15-24.

100. Selyanko AA, Brown DA. (1993) Effects of membrane potential and muscarine on potassium M-channel kinetics in rat sympathetic neurones. *J Physiol.* 472:711-724, 1993

101. Hadley JK, Noda M, Selyanko AA, Wood IC, Abogadie FC, Brown DA. (2000).Differential tetraethylammonium sensitivity of KCNQ1-4 potassium channels. *Br J Pharmacol.* 129: 413-415.

102.. Hadley JK, Passmore GM, Tatulian L, Al-Qatari M, Ye F, Wickenden AD, Brown DA. (2003). Stoichiometry of expressed KCNQ2/KCNQ3 channels and subunit composition of native ganglionic M-channels deduced from block by tetraethylammonium (TEA). *J Neurosci.* 23: 5012-5019.

103. Shah MM, Mistry M, Marsh SJ, Brown DA. Delmas P. (2002) Molecular correlates of the M-current in cultured rat hippocampal neurons. *J Physiol*. 544: 29-37

104. Selyanko AA, Delmas P, Hadley JK, Tatulian L, Wood IC, Mistry M, London B, Brown DA. (2002) Dominant-negative subunits reveal potassium channel families that contribute to M-like potassium currents. *J Neurosci.* 22: RC212.

105. Selyanko AA, Hadley JK, Wood IC, Abogadie FC, Delmas, P, Buckley NJ, London B. Brown DA. (1999) Two types of K^+ channel subunit, *Erg1* and *KCNQ2/3*, contribute to the M-like current in a mammalian neuronal cell. *J.Neurosci.* 19: 7742-7756.

106.Robbins J, Passmore GM, Abogadie FC, Reilly JM, Brown DA (2013) Effects of KCNQ2 gene truncation on M-type Kv7 potassium currents. . *PLoS One.* 8:e71809. doi: 10.1371/journal.pone.0071809.

107. Selyanko AA, Stansfeld CE and Brown DA. (1992). Closure of potassium M-channels by muscarinic acetylcholine-receptor stimulants requires a diffusible messenger. *Proc Roy Soc Ser B.* 250: 119-125.

108. Marrion NV. (1993). Selective reduction of one mode of M-channel gating by muscarine in sympathetic neurons. *Neuron* 11: 77-84

109. Marrion NV, Smart TG, Marsh SJ, Brown DA. (1989) Muscarinic suppression of the M-current in the rat sympathetic ganglion is mediated by receptors of the M1-subtype. *Br J Pharmacol*. 98:557-573.

110.. Delmas P, Brown DA. (2005) Pathways modulating neural KCNQ/M (Kv7) potassium channels. *Nat Rev Neurosci.* 6: 850-862.

111. Larrabee MG, Leicht WS.(1965) Metabolism of phosphatidyl inositol and other lipids in active neurones of sympathetic ganglia and other peripheral nervous tissues. The site of the inositide effect.*J Neurochem*. 12:1-13

112. Del Rio E, Bevilacqua JA, Marsh SJ, Halley P, Caulfield MP. (1999). Muscarinic M_1 receptors activate phosphoinositide turnover and Ca^{2+} mobilization in rat sympathetic neurones, but this signalling pathway does not mediate M-current inhibition. *J Physiol.* 520: 101-111

113. Winks JS, Hughes S, Filippov AK, Tatulian L, Abogadie FC, Brown DA, Marsh SJ (2005). Relationship between membrane phosphatidylinositol-4,5-bisphosphate and receptor-mediated inhibition of native neuronal M channels. *J. Neurosci*. 25: 3400-3413, 2005

114. Caulfield MP, Jones S, Vallis Y, Buckley NJ, Kim GD, Milligan G, Brown DA. (1994)Muscarinic M-current inhibition via G alpha q/11 and alpha-adrenoceptor inhibition of Ca2+ current via G alpha o in rat sympathetic neurones. *J Physiol.* 477: 415-422.

115. Haley JE, Abogadie FC, Delmas P, Dayrell M, Vallis Y, Milligan G, Caulfield MP, Brown DA, Buckley NJ. (1998) The alpha subunit of Gq contributes to muscarinic inhibition of the M-type potassium current in sympathetic neurons. *J Neurosci.* 18: 4521-4531.

116. Robbins J, Marsh SJ, Brown DA. (2006) Probing the regulation of M(Kv7) channels in intact neurons with membrane-targeted peptides. *J Neurosci* 26:7950-7961.

117. Delmas P, Abogadie FC, Dayrell M, Haley JE, Milligan G, Caulfield MP, Brown DA, Buckley NJ. (1998) G-proteins and G-protein subunits mediating cholinergic inhibition of N-type calcium currents in sympathetic neurons. *Eur J Neurosci*. 10: 1654-1666.

118. Haley JE, Delmas P, Offermanns S, Abogadie FC, Simon MI, Buckley NJ, Brown DA. (2000). Muscarinic inhibition of calcium current and M current in Galpha q-deficient mice. *J Neurosci*. 20: 3973-3979.

119. Haley JE, Abogadie FC, Fernandez-Fernandez JM, Dayrell M, Vallis Y, Buckley NJ, Brown DA. (2002). Bradykinin, but not muscarinic, inhibition of M-current in rat sympathetic ganglion neurons involved phospholipase C-β4. *J Neurosci* 20: RC105, 1-5.

120. Selyanko AA, Brown DA. (1996) Intracellular calcium directly inhibits potassium M channels in excised membrane patches from rat sympathetic neurons. *Neuron*. 16:151-162.

121. Gamper N, Shapiro MS. (2003). Calmodulin mediates Ca^{2+}-dependent modulation of M-type K^+ channels. *J. Gen. Physiol*. 122: 17–31.

122. Cruzblanca H, Koh DS, Hille B.(1998) Bradykinin inhibits M current via phospholipase C and Ca2+ release from IP3-sensitive Ca2+ stores in rat sympathetic neurons. *Proc Natl Acad Sci U S A*. 95: 7151-7156.

123. Delmas P, Wanaverbecq N, Abogadie FC, Mistry M, Brown DA. (2002).Signaling microdomains define the specificity of receptor-mediated InsP(3) pathways in neurons. *Neuron.* 34: 209-220.

124. Hughes S, Marsh SJ, Tinker A, Brown DA. (2007) PIP(2)-dependent inhibition of M-type (Kv7.2/7.3) potassium channels: direct on-line assessment of PIP(2) depletion by Gq-coupled receptors in single living neurons. *Pflugers Arch.* 455, 115-124.

125.Hoshi N, Langeberg LK, Scott JD. (2005). Distinct enzyme combinations in AKAP signalling complexes permit functional diversity.*Nat Cell Biol*. 7:1066-1073

126. Hoshi N, Zhang JS, Omaki M, Takeuchi T, Yokoyama S, Wanaverbecq N, Langeberg LK, Yoneda Y, Scott JD, Brown DA, Higashida H. (2003). AKAP150 signaling complex promotes suppression of the M-current by muscarinic agonists. *Nat Neurosci.* 6: 564-571

127. Kosenko A, Kang S, Smith IM, Greene DL, Langeberg LK, Scott JD, Hoshi N. (2012) Coordinated signal integration at the M-type potassium channel upon muscarinic stimulation. *EMBO J.* 31:3147-3156.

128. Huang CL, Feng S, Hilgemann DW.(1998) Direct activation of inward rectifier potassium channels by PIP2 and its stabilization by Gbetagamma. *Nature*. 391:803-806

129.. Suh BC, Hille B. (2002). Recovery from muscarinic modulation of M current channels requires phosphatidylinositol 4,5-bisphosphate synthesis. *Neuron*. 35: 507-520.

130.Zhang H, Craciun LC, Mirshahi T, Rohacs T, Lopes CM, Jin T, Logothetis DE. (2004). PIP(2) activates KCNQ channels, and its hydrolysis underlies receptor-mediated inhibition of M currents. *Neuron*. 37: 963-975.

131. Hille B, Dickson E, Kruse M, Falkenburger B. (2014). Dynamic metabolic control of an ion channel. *Prog Mol Biol Transl Sci*. 123:219-247.

132. Kruse M, Vivas O, Traynor-Kaplan A, Hille B. (2016). Dynamics of phosphoinositide-dependent signaling in sympathetic neurons. *J Neurosci.* 36:1386-1400.

133. Telezhkin V, Brown DA, Gibb AJ (2012). Distinct subunit contributions to the activation of M-type potassium channels by PI(4,5)P_2. *J. Gen.Physiol*. 140:41-53.

134. Li Y, Gamper N, Hilgemann DW, Shapiro MS. (2005). Regulation of Kv7 (KCNQ) K+ channel open probability by phosphatidylinositol 4,5-bisphosphate. *J Neurosci*. 25:9825-9835.

135.Houamed KM, Bilbe G, Smart TG, Constanti A, Brown DA, Barnard EA, Richards BM. (1984) Expression of functional GABA, glycine and glutamate receptors in Xenopus oocytes injected with rat brain mRNA. *Nature.* 310:318-321

136. Brown DA, Docherty RJ, Halliwell JV (1984). The action of cholinomimetic substances on impulse conduction in the habenulointerpeduncular pathway of the rat in vitro. *J Physiol*.353:101-109. [Authors in alphabetical order as per journal style]

137.Marsh SJ, Stansfeld CE, Brown DA, Davey R, McCarthy D.(1987). The mechanism of action of capsaicin on sensory C-type neurons and their axons in vitro. *Neuroscience.* ;23:275-289.

138. Marsh,SJ, Trouslard J, Leaney JL, Brown DA. (1995). Synergistic regulation of a neuronal chloride current by intracellular calcium and muscarinic receptor activation: a role for protein kinase C. *Neuron,* 15: 729-737.

139. Filippov, A.K., Couve, A., Pangalos, M.N., Walsh, F.S., Brown, D.A. & Moss, S.J. (2000). Heteromeric assembly of $GABA_BR1$ and $GABA_BR2$ receptor subunits inhibits Ca^{2+} current in sympathetic neurons. *J.Biol.Chem.*, 20: 2867-2874.

140. Pan, Z., Selyanko, A.A., Hadley, J.K., Brown, D.A., Dixon, J.E. & McKinnon, D. (2001). Alternative splicing of KCNQ2 potassium channel transcripts contributes to the functional diversity of M-currents. *J. Physiol.*, 531: 347-358.

141. Fernandez-Fernandez, J.M., Abogadie, F.C., Milligan, G., Delmas, P. & Brown, D.A. (2001). Multiple pertussis toxin-sensitive G proteins can couple receptors to GIRK channels in rat sympathetic neurones when heterologously-expressed, but only native G_i proteins do so *in situ. Eur. J. Neurosci.* 14: 283-292.

142. Tatulian L, Delmas P, Abogadie FC, Brown DA (2001). Activation of expressed KCNQ potassium currents and native neuronal M-type currents by the anti-convulsant drug retigabine. *J. Neurosci.* 21,5535-5545

143. Delmas P, Nomura H, Li X, Lakkis M, Luo Y, Segal Y, Fernandez-Fernandez JM, Harris P, Frischauf A-M, Brown DA, Zhou J. (2002). Constitutive activation of G-proteins by polycystin-1 is antagonized by polycystin-2. *J. Biol. Chem.*, 277, 11276-11283.

144. Wanaverbecq N, Marsh SJ, Al-Qatari M, Brown DA. (2003). The plasma membrane calcium ATPase as a major mechanism for intracellular calcium regulation in neurons from the rat superior cervical ganglion. *J.Physiol.*, 550, 83-101

145. Allen TG, Abogadie FC, Brown DA. (2006). Simultaneous release of glutamate and acetylcholine from single magnocellular "cholinergic" basal forebrain neurons. *J Neurosci.* 26: 1588-1595

146. Shah, MM, Migliore M, Valencia I, Cooper EC, Brown DA. (2008) Functional significance of axonal Kv7 channels in hippocampal pyramidal neurons. *Proc Natl Acad Sci U S A* 105**:** 7869-7874.

147 Passmore GM, Reilly JM, Thakur M, Keasberry VN, Marsh SJ, Dickenson AH, Brown DA (2012) Functional significance of M-type potassium channels in nociceptive cutaneous sensory endings. *Front. Mol. Neurosci.* **5**:63. doi: 10.3389/fnmol.2012.00063

Fig 1

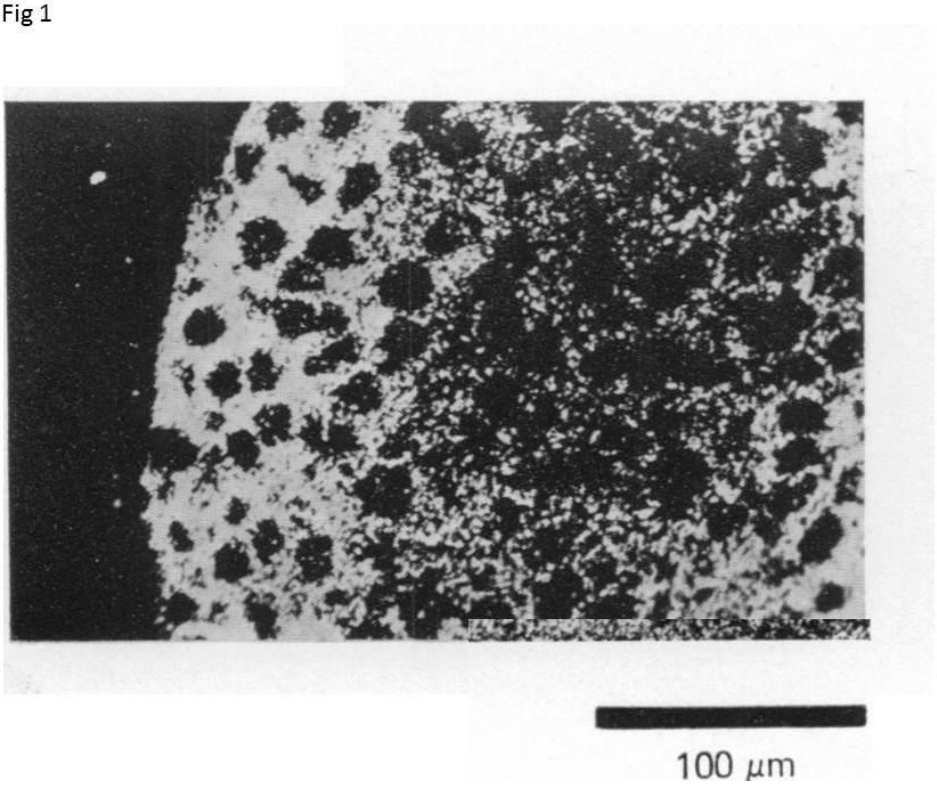

Fig.1. Autoradiograph of ^{3}H-GABA uptake into a rat superior cervical sympathetic ganglion. An isolated ganglion was incubated in a physiological saline solution containing 0.4 μM ^{3}H-GABA for 30 min, rinsed in non-radioactive solution for 30 min, then processed and sectioned at 5 μm thickness as described in [**35**]. The picture shows a darkfield image under reflected light so the silver grains show up white. Uptake is into neuroglial cells only, so neurons show up as black holes. Adapted from ref [**35].**

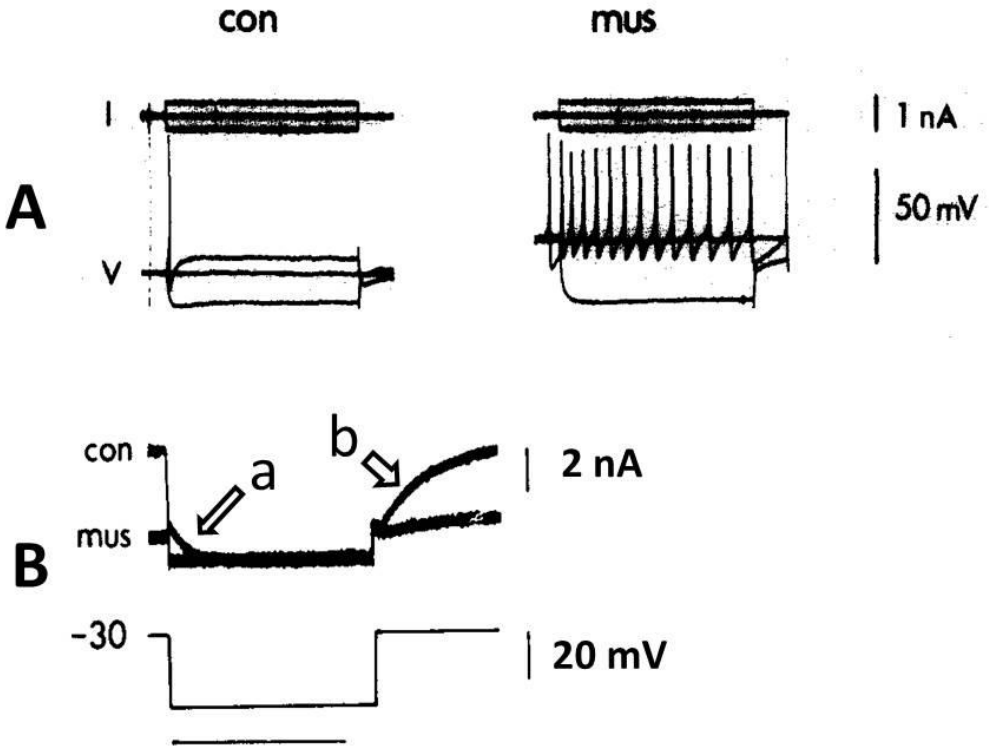

Fig.2. Effect of muscarine (mus, 10 µM) on A a rat sympathetic neuron under current-clamp and B a bullfrog sympathetic neuron under voltage-clamp.

- **A.** Recordings from a neuron in an isolated rat superior cervical sympathetic neuron superfused at 30°C and impaled with a single microelectrode filled with 4M K^+ acetate. Records show voltage responses (**V**) to 1s +/- 0.4 nA current injections (**I**). Muscarine depolarized the cell, increased its input resistance (larger voltage response to hyperpolarizing current) and induced repetitive action potential discharges during depolarizing current injection. (Adapted from [**57**])
- **B.** Superimposed recordings of current responses to 1 s voltage steps from -30 mV (holding potential) to -60 mV before (con = control) and after adding muscarine (mus) to a neuron in an isolated bullfrog lumbar sympathetic ganglion superfused at 22°C and impaled with two microelectrodes filled with 3M KCl (to record current and set voltage). Before adding muscarine, the current record (top) shows a steady outward current due to current flow through M-type K^+ channels. On stepping to -60 mV these channels slowly close, giving the inward relaxation marked **a,** followed by the outward relaxation **b** as the M-channels re-open on returning to -30 mV. Channel closure (rather than opening) is shown by the fall in instantaneous current between stepping from -30 mV and stepping back from -60 mV. Muscarine closed the M-channels, generating an inward current at the holding potential, and abolishing M-channel closure and reopening currents. (Experiment with Paul Adams, from Brown (1983). *Trends in Neurosci*, 6:302-306).

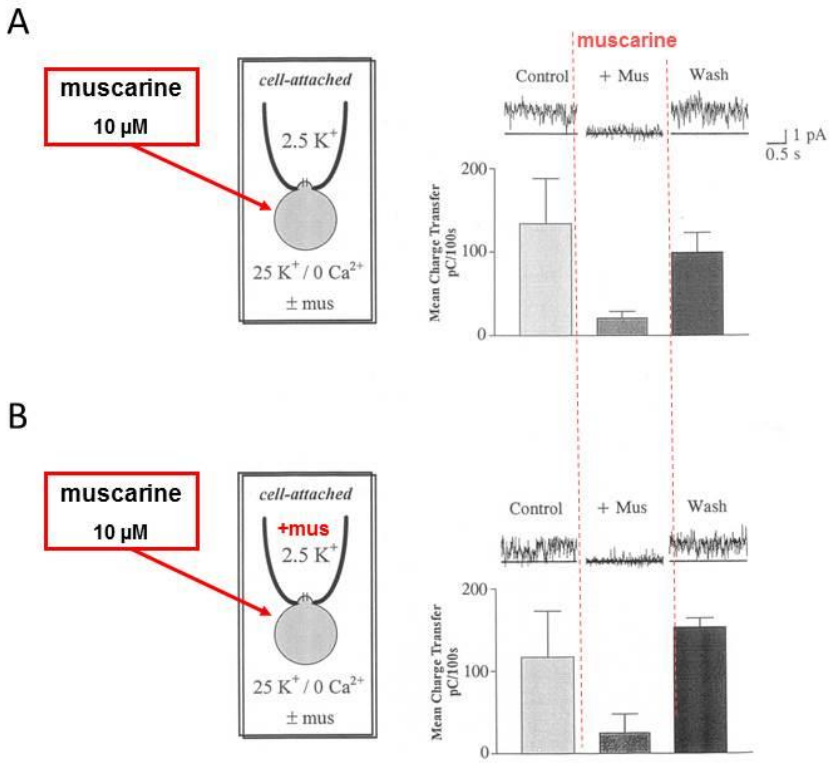

Fig.3 Muscarine uses a mobile messenger system to close M-channels.

Records (right) show M-channels activity recorded from two rat sympathetic neurons using cell-attached patch pipettes. The cell was incubated in a solution containing 25 mM KCl to fix the membrane potential at -30 mV) and O mM $CaCl_2$ (to prevent Ca-loading). The membrane potential under each patch-pipette was set to 0 mV to open M-channels, giving the continuous channel activity seen in the upper control records in A and B. Bars below records show average (+ S.E.M, n=8 (A), 4(B)), channel current activity in picocoulombs (pC)/100sec. In cell **A** muscarine (mus; 10 µM) added to the solution bathing the cell outside the patch-pipette caused the channels inside the pipette to close (middle upper record in A) which re-opened (right-hand records) when the muscarine was washed out of the superfusion fluid. In cell **B** muscarine was added to the pipette solution *before* attaching the pipette to the cell membrane. Nevertheless, the channels still opened on depolarizing the membrane patch and were closed as in A when muscarine was superfused over the cell membrane outside the pipette. Adapted from [**107**]