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Serotonin and the Australian Connection: The Science and the People

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ABSTRACT: This contribution to "Putting the pieces together: Proceedings from the International Society for Serotonin Research (aka Serotonin Club)" encapsulates a brief history of serotonin beginning with its discovery in 1946 by Maurice Rapport, Arda Green, and Irvine Page. The first 40 years of serotonin research culminated in the inaugural Serotonin Club meeting held on Heron Island, Australia, in 1987. In light of the silver anniversary of the Serotonin Club and its Australian beginnings, it is timely to highlight some of the contributions made to serotonin research by Australian scientists, which I shared with participants at the 2012 meeting of the Serotonin Club, in Montpellier, France as the honoree of the Maurice Rapport Lectureship.

KEYWORDS: *Serotonin (5-hydroxytryptamine; 5-HT), smooth muscle, cardiac muscle, 5-HT receptor nomenclature*

Researchers first noticed the effects of serotonin in 1868 regarding a substance released from platelets during the blood clotting process. This substance was given various names prior to "serotonin". Maurice Rapport's analysis of "serum vasoconstrictor" commenced in 1946 at the Cleveland Clinic Foundation. Rapport, who completed his Ph.D. in 1945 in organic chemistry at Caltech, began his investigation of serotonin with colleagues Arda Green and Irvine H. Page (Director of the Cleveland Clinic). Rapport carried out his analysis in phases: detection, isolation, characterization as a purified substance, and, finally, the identification of the chemical structure of 5-hydroxytryptamine (5-HT). Three seminal publications from Rapport, Green, and Page in 1948[1−2] and another from Rapport in 1949[4] described the nature of serotonin and its chemical structure. These findings enabled other early researchers to understand pharmacologic effects of serotonin on smooth and cardiac muscle.

The earliest investigations of serotonin pharmacology began when Rapport sent a sample of serotonin to John Gaddum and Hans Heller (Great Britain). In April 1951, Vittorio Erspamer (Italy) also requested a sample from Rapport. Erspamer had studied "enteramine" from chromaffin cells in the gastrointestinal tract. The outcome was that serotonin and enteramine were realized to be identical, except for the results of colorimetric tests using different chromatography papers, with differences being due to the papers themselves.[5,6]

Rapport also sent a sample of serotonin to George Reid and Michael Rand (University of Melbourne, Australia) in November 1951. However, before the sample arrived,[7] Reid and Rand had already published five papers on the pharmacological actions of serotonin.[8−12] By following the methods published by Rapport et al. over the period of 1946−1949, Reid and Rand had prepared serotonin by themselves. Their purified serotonin produced the following effects *in vivo*: an increase, then a longer decrease in arterial blood pressure in cats, dogs, and rabbits; increased pulmonary blood pressure, apnea, bronchoconstriction, bladder constriction, pupil constriction, and increased nictitating membrane activity. In *in vitro* preparations from cats, dogs, and sheep, serotonin caused arterial strips to contract; likewise, serotonin caused rat and guinea pig uterus preparations to contract. It also had similar effects on guinea pig and rabbit intestine preparations (Figure 1).

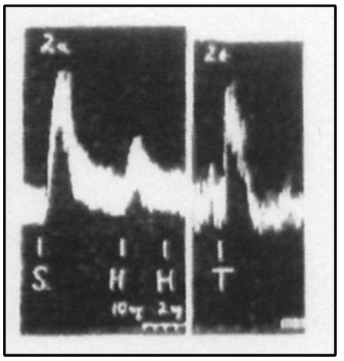

Figure 1. Two recordings of rabbit jejunum contraction *in vitro*: S, 0.4 mL of rabbit serum; H, 10 and 2 μg histamine; and T, thrombotonin (i.e., serotonin). [Reprinted with permission from ref 11.]

BRIEF BIOGRAPHIES OF GEORGE REID AND MICHAEL J. RAND

George Reid was born in 1915 in Ballarat, Victoria, Australia, and graduated with a degree in medicine from the University of Melbourne in 1936. He was third in his class of 103 students. In 1939, he joined the staff of the Walter and Eliza Hall Institute of Medical Research. In 1942, he enlisted in the Australian Infantry Forces and was discharged in 1945. He was then appointed as Senior Lecturer at the University of Melbourne in the Department of Physiology. In 1948, he was awarded a

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Rockefeller fellowship to conduct research in pharmacology at Oxford University, and in clinical medicine at Hammersmith Hospital in London. Reid published numerous medical research articles, including papers on vasoconstrictor substances in serum that influenced the clotting of blood. He was awarded the degree of Doctor of Science (Melbourne) in early 1952. Dr. Reid suffered from malignant hypertension resulting in a stroke in 1948. He underwent a bilateral thoracolumbar sympathectomy (the only treatment available at that time). Unfortunately, 4 years later, at the age of 37, he suffered a second and fatal stroke. Clinical trials on hexamethonium to control hypertension were initiated a year later.

Michael J. Rand was born in 1927 in Mildenhall, Suffolk, U.K. His mother migrated to Australia with her two sons and settled in Melbourne. Rand gained a Bachelor degree in Biological Sciences at the University of Melbourne, and was then accepted into a Master of Science program in the University of Melbourne's Department of Physiology. George Reid supervised his research. By then, Rand had become an Australian citizen. The death of Reid was a significant factor in Rand's decision to concentrate his research in the field of autonomic pharmacology. He completed his Ph.D. in Pharmacology at the University of Sydney in 1957, supervised by Professor Roland Thorpe. Rand secured an appointment as Departmental Demonstrator to work with the eminent pharmacologist Professor J. H. Burn at the Department of Pharmacology, Oxford University. Rand was later appointed to the London School of Pharmacy. In 1964, Rand took leave to work with Professor Geoffrey Burnstock at the University of Melbourne. In 1965, Rand was appointed as the Chair of Pharmacology at the University of Melbourne and remained there until his retirement in 1992. Rand continued his research on a part-time basis through an arrangement at RMIT University in Melbourne. This was made possible by Professor David Story, who was the first Ph.D. student during Rand's tenure as Chair of Pharmacology. Dr. Rand died in 2002.

On a personal note, I completed my Diploma in Pharmacy in 1965. As a student in 1964, I had the privilege of listening to a lecture from Michael Rand. His lecture made me decide that I needed to study more physiology and pharmacology. I enrolled at the University of Melbourne in 1967 to study with Dr. Rand. I gained a Bachelor of Science (Honors first class) and then completed a Ph.D. on β-adrenoceptor blockers in 1974. Rand was an outstanding mentor for me during those years, and I enjoyed his lifelong friendship. Likewise, my Ph.D. supervisor and mentor, Colin Raper (London School of Pharmacy), taught me pharmacology, and we remain friends. In 1973, when the majority of my Ph.D. research was finished, Rand arranged my first postdoctoral appointment as a National Health and Medical Research Council (NHMRC) Senior Research Officer, School of Medicine, University of NSW, Sydney. Here, my research shifted to the study of the role of serotonin in migraine. I worked closely with Professor James Lance, an internationally known neurologist specializing in migraine, to investigate internal and external carotid artery responses to humoral agents in anesthetized *Macaca nemestrinas* monkeys.

MIGRAINE RESEARCH AND SEROTONIN

Little information existed on the effects of humoral agents, so my research carried out in Sydney was aimed at the reactivity of the cerebral vasculature, particularly the common and external carotid arteries, using electromagnetic flowmeter probes (Figure 2).

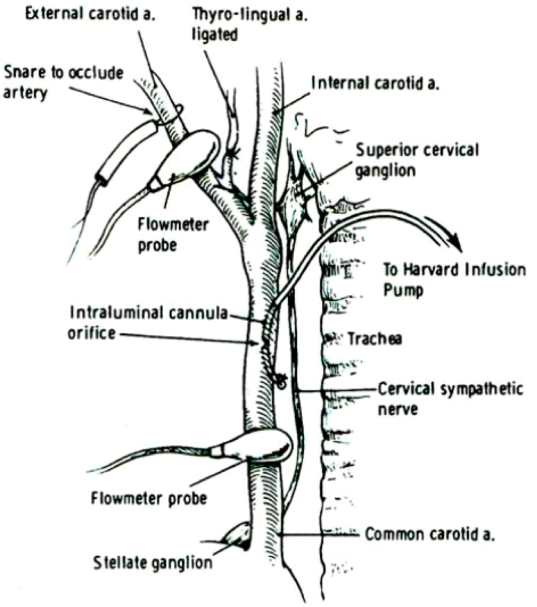

Figure 2. Diagram of the common, external, and internal carotid arteries, flowmeter probes, intraluminal cannula and orifice, snare to occlude the artery, and other landmarks, for anesthetized *Macaca nemestrinas* monkeys. [Reprinted with permission from ref 13.]

Vasoactive agents were tested using electromagnetic flowmeter probes. Both the internal and external carotid arteries were constricted by serotonin and PGF_{2a}, and dilated by bradykinin, histamine, and acetylcholine. Noradrenaline and adrenaline constricted the external carotid but not the internal carotid, and PGE_1 dilated the external carotid.[13] Investigation was further carried out on the effects of methysergide, pizotifen, and ergotamine on external and internal carotid vascular resistance (ECVR, ICVR), as well as the responses of these arteries to 5-HT and noradrenaline. Methysergide reduced serotonin-induced ECVR and ICVR, and potentiated the effects of noradrenaline on ECVR but not ICVR. Pizotifen reduced the effects of serotonin on ECVR, but not ICVR. Ergotamine reduced the effects of serotonin and noradrenaline and was also an intrinsic constrictor.[14]

In 1978, I was appointed to a lectureship in the Department of Pharmacology, University of Sydney. In those first few years as a junior lecturer, I began teaching and gradually setting up research projects and gained my first independent NHMRC 3-year research grant (1980–1982). The focus of this project was to begin to devise standard nomenclature for serotonin and its receptors. As pharmaceutical laboratories developed new agonists and antagonists with which to study the serotonin system, it became clear that serotonin produced its various physiological effects via actions at multiple receptors. Thus, the use of "5-HT" soon replaced "serotonin" as a first step to standardizing nomenclature for its numerous receptors.

We used the 5-HT "D" antagonist, ketanserin, in 1982 for *in vitro* studies on smooth muscle. However, in *in vivo* 5-HT vasodilatation studies in anesthetized dogs, we noted the hypotensive effects the hypotensive effects of intravenous (iv) ketanserin (0.1–4 mg/g). Ketanserin did not inhibit common carotid vasoconstrictor responses to intraarterial (ia) noradrenaline, preganglionic sectioned cervical sympathetic nerve, or iv nicotine. Systemic constrictor responses to iv nicotine (producing sympathetic activation via CNS and ganglionic stimulation) and common carotid artery occlusion were

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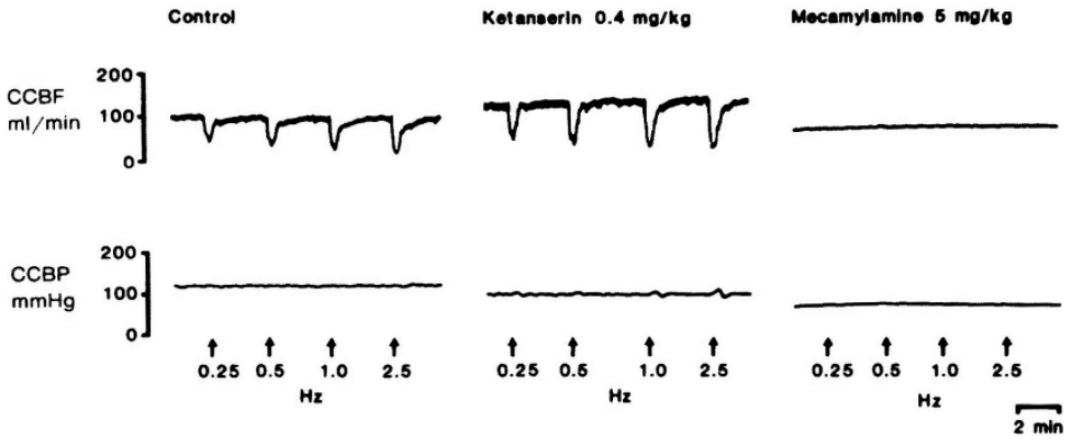

Figure 3. Traces from a typical experiment showing common carotid blood flow (CCBF) and common carotid blood pressure (CCBP) responses to cervical sympathetic nerve stimulation at the frequencies indicated, initially (Control), after administration of ketanserin (0.4 mg/kg, iv), and after the subsequent administration of mecamylamine (5 mg/kg, iv). [Reprinted with permission from ref 15.]

inhibited by ketanserin. Together, these results suggested that the hypotensive action of ketanserin had a CNS origin (Figures 3 and 4).[15]

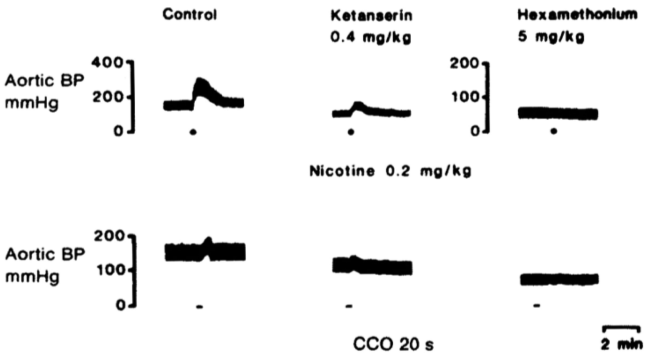

Figure 4. Traces from a typical experiment showing systemic blood pressure (aortic BP) responses to nicotine (0.2 mg/kg, iv) and unilateral common carotid occlusion (CCO) for 20 s, initially (Control), after administration of ketanserin (0.4 mg/kg, iv), and after the subsequent administration of hexamethonium (5 mg/kg, iv). [Reprinted with permission from ref 15.]

The vasodilator effects of serotonin had been known since the 1950s (*in vivo* and *in vitro*), but the underlying mechanisms remained unknown until Michael McGrath found in 1977 that serotonin had a presynaptic inhibitory effect on sympathetic nerve activity in canine saphenous and tibial artery preparations *in vitro*.[16] McGrath, a University of Sydney medical graduate, was sponsored for a Traveling Fellowship by the Royal Australasian College of Physicians and the Mayo Clinic, to work in John Shepherd's laboratory.

Our small group was the first to confirm these findings *in vivo* in 1985.[17] Studying femoral arterial circulation in anesthetized dogs, we tested the possibility that a presynaptic inhibitory effect of 5-HT on adrenergic function could produce the vasodilatory actions of 5-HT. Femoral vascular resistance (FVR) was recorded as cumulative dose–response curves. In control responses, ia 5-HT and acetylcholine (ACH) produced marked vasodilatation. After ganglion blockade (mecamylamine) or ornipressin (vasoconstrictor), 5-HT dilatation was abolished, but the effect of ACH was unchanged (Figure 5). From these studies, we showed that 5-HT dilatation relies on

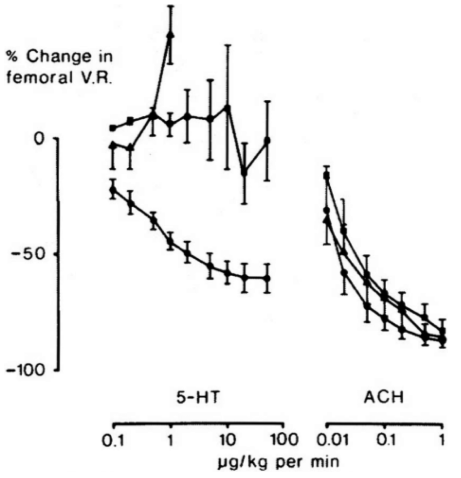

Figure 5. Mean log dose–response curves for the femoral vasodilatation effect of 5-HT and acetylcholine (ACH), before (control: circle) and after ganglion block with mecamylamine (5 mg/kg, iv; square) and after restoration of femoral vascular tone by the simultaneous ia infusion of ornipressin (0.005–0.001 IU/kg per min: triangle). In each experiment, femoral vascular resistance values were expressed as percentage changes from the preinfusion femoral vascular resistance levels for 5-HT and ACH. Responses to all doses of 5-HT after ganglion block alone and after restoration of femoral vascular tone were significantly different from the control responses ($P < 0.05$); responses to ACH did not differ significantly from the corresponding control responses. [Reprinted with permission from ref 17.]

the inhibition of adrenergic function.[17] It took a decade or more to identify the 5-HT receptors in question: 5-HT1A, 1B, and 1D.

■ 5-HT RECEPTOR NOMENCLATURE

From the early 1980s, the expansion in the numbers of new drugs affecting 5-HT function triggered a thriving approach to classifying and understanding the function of 5-HT receptors. By 1986, a commentary article was authored by Philip Bradley, Guenter Engel, Was Feniuk, John Fozard, Patrick Humphrey, Derek Middlemiss, Ewan Mylecharane, Brian Richardson, and Pramod Saxena.[18] At this stage, serotonin receptors were classified as 5-HT1-like, 5-HT2, and 5-HT3 receptors, each with defined functional agonist and antagonist effects on

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neuronal inhibition, smooth muscle contraction or relaxation, and tachycardia, in various *in vitro* and *in vivo* tissue preparations and cells.

Two examples of the *in vivo* effects of 5-HT receptors in cat cardiac muscle and cat smooth muscle in the urinary bladder were investigated in the laboratory of Pramod Saxena in Rotterdam, where I had been invited for my first sabbatical in 1984. (There were several subsequent visits between Rotterdam and Sydney to work with Pramrod.) Investigation of heart rate in anesthetized cats revealed that the 5-HT1-like agonist, 5-carboxamidotryptamine, induced tachycardia, similar to that elicited by 5-HT, suggesting that this effect may be mediated by 5-HT1-like receptors. Tachycardia elicited by 5-HT was also found to be mediated by 5-HT2 receptors (blocked by methysergide, ketanserin, ritanserin, pizotifen, and other drugs). Bradycardia elicited by 5-HT was mediated by 5-HT3 receptors and blocked by MDL 72222.[19] In the anesthetized cat urinary bladder preparation, a biphasic contraction elicited by 5-HT was first mediated by 5-HT3 receptors and blocked by MDL 72222. The second phase of this contraction was mediated by 5-HT2 receptors (blocked by ketanserin, methysergide, and cyproheptidine).[20]

Pharmacological Reviews published a seminal classification of 5-HT receptors in 1994, under the auspices of the International Union of Pharmacology (IUPHAR).[21] The authors were Daniel Hoyer, David Clarke, John Fozard*, Paul Hartig, Graeme Martin, Ewan Mylecharane*, Pramod Saxena*, and Patrick Humphrey* (*authors who contributed to the 1986 Bradley et al. review). The earlier receptor classification of 5-HT1-like receptors was expanded greatly by 1994. Furthermore, 5-HT1, 5-HT2, 5-HT3, and 5-HT4 receptors were fully confirmed from experimental evidence, as were receptor subtypes including 5-HT1A, 1B, 1D, 1E, 1F and 5-HT 2A, 2B, 2C. Cloned 5-ht5, 5-ht6, and 5-ht7 receptors were not yet fully confirmed yet via functional, signal transduction, and structural criteria. According to the ISI Web of Knowledge in June 2012, citations for the Bradley et al. article were >1200 and those for the Hoyer et al. article were >2300.

FOUNDING THE SEROTONIN CLUB

From July 1985, with Paul Vanhoutte at the helm, three premeetings were organized in 1986 (April, St. Louis, MO, U.S.A.; November, Washington, DC, U.S.A.; and December, London, U.K.). By September 1987, 324 members had joined the Serotonin Club! The official inaugural meeting was held during the 1987 IUPHAR Congress in Sydney, Australia, at a dinner on Friday, August 28, 1987. This was a formal meeting to appoint the inaugural officers and to approve the constitution, a Nomenclature Committee, and financial details. A few days later, the first scientific conference was held by the Serotonin Club over 3 days (September 4–6, 1987) with participants traveling from Sydney to Heron Island, Queensland. This first conference was an official Satellite meeting of the 10th IUPHAR Congress, Sydney, August 1987. The inaugural Serotonin Club meeting was convened by Ewan Mylecharane, James Angus, Ivan de la Lande, and Patrick Humphrey, with an Organizing Committee comprising John Fozard, Peter Howe, Jennifer Kennedy, Ralph Purdy, Brian Richardson, Pramod Saxena, Paul Vanhoutte, Jan Van Nueten, and the other meeting organizers. In the wake of its highly successful first 25 years, may the Serotonin Club continue for another 25 years and beyond.

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Notes

The authors declare no competing financial interest.

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