A pharmacological analysis of the mode of action of serotonin (5-hydroxytryptamine) upon the guinea-pig ileum

M. Rocha e Silva, J.R. Valle & Z.P. Picarelli

Commentary by

G.R. Martin

Rocha e Silva, Valle and Picarelli justly take credit for presenting the first phamacological evidence to suggest that 5-hydroxytryptamine (5-HT) can evoke excitation of autonomic neurons. The experiments described in their paper, published only 2 years after the monoamine was first synthesized (Hamlin & Fischer, 1951; Speeter *et al.*, 1951), bear all the hallmarks of classic, analytical pharmacology, enabling these authors correctly to deduce that, in isolated guinea-pig ileum, a prominent part of the smooth muscle stimulant effects of 5-HT is mediated indirectly *via* excitation of postganglionic, parasympathetic myenteric neurons. This was the first robust evidence for such an action of 5-HT and undoubtedly set Picarelli, subsequently working with Gaddum, on the path to what is arguably the most influential scientific paper in the 5-HT field to date: that describing the first sub-division of 5-HT receptors into neuronal 'M' and non-neuronal 'D' subtypes (Gaddum & Picarelli, 1957).

The insight and skill with which Rocha e Silva and colleagues conducted their studies is apparent when considered in the context of the information available to them at that time. In the guise of either vasotonin (serotonin: Rapport *et al.*, 1948) or enteramine (Erspamer & Asero, 1952), 5-HT was already known to be a powerful spasmogen in isolated tissue preparations. However, studies *in vivo* revealed complex cardiovascular actions that were clearly incompatible with a simple spasmogenic action. Page & McCubbin (1952) coined the term 'amphibaric' to describe the ability of 5-HT to produce its triphasic effect on blood pressure, comprising an immediate rapid fall, followed by a brisk rise and finally a more prolonged fall. Rocha e Silva seized upon Page's (1952) observation that the initial fall in blood pressure, attributable largely to the von Bezold-Jarisch reflex, was abolished by atropine implying that a part of the activity of 5-HT was "...parasympathetic or cholinergic in nature." It is clear from their paper (see Introduction and Addendum) that their own studies had firmly convinced the authors that 5-HT contractions of the isolated ileum exhibited similar 'cholinergic' qualities, since, in their hands, 5-HT effects were reliably blocked by atropine (~0.1μM). This was probably *the* key observation that steered them towards an evaluation of potential 5-HT interactions with myenteric neurons. However, this prejudice also presented the group with some intriguing dilemmas. Firstly, it obliged the authors to confront a long-established dogma which considered that parasympathetic nerve activation in the gut was not atropine-sensitive (Bayliss & Starling, 1899; Dale & Gaddum, 1930; Vogt, 1943). Secondly, two independent reports had appeared only two months earlier in which contractions of the guinea-pig ileum to 5-HT were shown to be atropine-insensitive (0.1–1μM: Gaddum, 1953; Feldberg & Toh, 1953). In the face of these data, from some of the most eminent pharmacologists of the era, the proposal that 5-HT might act indirectly in the gut by stimulating the release of acetylcholine from myenteric neurons must have been looked upon as apostasy!

Interestingly, two articles published as Proceedings of the Physiological Society in March and June of 1953 could have been used by Rocha e Silva *et al.* as support for their proposal, but neither paper was cited by the authors. The first, by Sinha & West (1953), reported that 5-HT contractions of guinea-pig ileum were inhibited by various local anesthetics, including cocaine. The sec-

ond, by Robertson (1953), directly confirmed the authors' own observations that 5-HT contractions were blocked by low concentrations of atropine (0.01-0.1μM). This apparently inconsistent effect of atropine must have been perplexing. So much so, in fact, that with due deference to Gaddum (1953) and Feldberg & Toh (1953), Rocha e Silva and colleagues added an Addendum to their paper describing further experiments which again confirmed the ability of atropine to block 5-HT contractions in the ileum. They went on to suggest that, in principle, differences in buffer pH might account for the variable sensitivity of responses to atropine in this tissue. Thankfully, the absence of a more tangible explanation seems not to have been an overriding issue for the authors.

Evidence reflecting the courage of the authors' conviction that a prominent action of 5-HT somehow involved parasympathetic excitation appears early in the Results section of their paper. They noted that the contractile response to 5-HT appeared qualitatively distinct from the response to either histamine or acetylcholine, but resembled that provoked by nicotine. This observation, coupled with the demonstration that serotonin produced homologous, but not heterologous (*vs.* acetylcholine or histamine) desensitisation of the tissue, prompted the authors' first important conclusion; namely, that 5-HT could not be acting directly at smooth muscle acetylcholine receptors. They therefore tested the possibility that, like nicotine, it acted instead to stimulate ganglia in the myenteric plexus. Exposure of tissues to moderate concentrations (8-80μM) of nicotine produced a transient contraction, after which responses to 5-HT, but not acetylcholine, bradykinin or histamine, were abolished. However, after repeated exposures to nicotine (120-160μM), contractions to 5-HT reappeared, i.e. they 'escaped' from inhibition by nicotine. This dual behavior of nicotine was widely recognized but not fully understood at the time. It is now known to result from an initial excitation of nitotinic receptors followed by a rapid desensitisation, resulting in transient ganglion stimulation followed by a depression of ganglionic transmission. Since the transient contractions evoked by nicotine were unaffected by a desensitizing concentration of 5-HT, Rocha e Silva and co-workers drew two further conclusions; firstly, that 5-HT could not be acting at the same site as nicotine, and secondly, that it could not be acting at the level of the ganglion. The fact that the ganglion blocking drug hexamethonium completely blocked responses to nicotine whilst leaving contractions to 5-HT unaffected, reaffirmed these conclusions.

Not content with these fundamental observations, the authors went on to offer an explanation for the behavior of nicotine in their studies which modified the prevailing view that refractoriness to nicotine resulted from 'metabolic exhaustion of the smooth muscle'. They speculated that moderate concentrations of nicotine might stimulate sympathetic ganglia within the myenteric plexus to provoke the release of noradrenaline which subsequently acted as a 'physiological antagonist' of 5-HT. The use of exogenously added adrenaline confirmed that this was plausible. Higher nicotine concentrations were envisaged to exhaust post-ganglionic stores of noradrenaline, accounting for the 'escape' phenomenon. With the wisdom of hindsight, these considerations, although perceptive, probably distracted the authors from a simpler explanation that would have led them directly to their ultimate conclusion. Had they understood that nicotine simply caused desensitisation block of ganglionic transmission, they would have been obliged at this point in their studies to conclude that 5-HT must be acting at post-ganglionic parasympathetic neurons to provoke the release of acetylcholine.

Instead, Rocha e Silva and coworkers reached this conclusion after conducting what might be considered the most far-reaching experiment described in their paper. They demonstrated that a low concentration of cocaine (2μM), sufficient to block the stimulatory effects of nicotine, also abolished contractions to 5-HT without affecting responses to either acetylcholine or histamine. In the words of the authors, this was "*...the key to explain the locus of action of [serotonin] upon the gut*", since according to their line of thinking, it left only two possible modes of action; either pre- or post-ganglionic parasympathetic neuro-excitation. Since 5-HT contractions were unaffected by ganglion block, they surmised that "*...the only indirect point of attack for serotonin would be the post-ganglionic fibres that can be blocked by cocaine*".

From a modern perspective, the elegant simplicity of the experiments described in this paper belies the complexity of 5-HT effects in the guinea-pig ileum. Of the fourteen currently recognized 5-HT receptor subtypes, five are functionally active in this tissue (Hoyer *et al.*, 1994). The pro-contractile smooth muscle 'D' receptor

described by Gaddum & Picarelli in 1957 is now known to be the 5-HT_{2A} receptor (Engel *et al.*, 1984), whereas in the tonically contracted ileum, 5-HT_7 receptors mediate direct smooth muscle relaxation (Feniuk *et al.*, 1983; Eglen *et al.*, 1994). In addition, three different 5-HT receptors modulate neurotransmitter release from guinea-pig myenteric neurons; 5-HT_{1A} heteroreceptors *inhibit* depolarization-evoked acetylcholine release (Fozard & Kilbinger, 1985; Kilbinger & Wolf, 1992), whereas 5-HT_3 and 5-HT_4 receptors both provoke transmitter release by neuroexcitation (Craig & Clarke, 1990; Kilbinger & Wolf, 1992). An additional neuronal 'orphan' receptor, 5-HT_{1P}, mediates slow excitatory synaptic potentials, the functional significance of which is unknown (Gershon *et al.*, 1985). In spite of this multiplicity of 5-HT receptors, under the conditions of their experiments (5-HT=0.8μM), Rocha e Silva *et al.* – as well as their contemporaries at the time - would have witnessed the effects only of 5-HT_3 and 5-HT_4 receptor activation. More recent studies indicate that the 5-HT_3 receptor, which equates to Gaddum & Picarelli's (1957) 'M' receptor, most likely accounted for the responses. Both receptors stimulate the release of acetylcholine, although functional evidence indicates that 5-HT_3 receptor-mediated contractions are also mediated, in part, by the concomitant release of Substance P (Buchheit *et al.*, 1985). This contemporary knowledge may explain the differences in sensitivity of 5-HT to atropine reported in the early studies with this tissue, since the relative contributions of acetylcholine and Substance P to 5-HT-induced contractions probably varies considerably with animal strain, age and experimental conditions.

If one was to be in any way critical of this paper, then it would be to question why Rocha e Silva *et al.* chose not to capitalize on earlier experiments by Ambache (1946), in which methods were described enabling directly- and indirectly-acting stimulants of isolated gut tissue to be distinguished. Simple manipulations, such as elevating the concentration of extracellular calcium or prolonged cooling, were shown to abolish the effects of drugs which act by parasympathetic neuroexcitation and could have provided rapid confirmation of 5-HT's indirect actions in the ileum. Perhaps the answer lies in the authors' emphasis on establishing that the effects of 5-HT were specific and independent of the receptor mechanisms known at that time. This they achieved whilst providing at the same time the first chemical lead, in cocaine, to selective neuronal 5-HT receptor antagonists. In failing to recognize the significance of this result, we too are culpable, since it preceded, by nearly 20 years, the studies of Fozard and colleagues which laid the foundations for the discovery of MDL 72222, the first in a generation of highly selective 5-HT_3 receptor antagonist drugs (see Fozard, 1989). In this regard, the paper by Rocha e Silva and colleagues deserves greater appreciation, not only as an outstanding example of rigorous pharmacology, but also as the forebear of work that has culminated in a novel class of drugs representing one of the most significant therapeutic advances of our time.

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