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Is Desensitization of Intestinal 5-Hydroxytryptamine Receptors an In-vitro Phenomenon?

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Abstract—The responses of proximal jejunum and distal ileum to successive applications of 5-hydroxytryptamine (5-HT) were examined in-vitro and in-vivo by measuring the electrical changes that reflect the stimulation of Cl^- secretion. In stripped intestinal sheets the second application of a maximal concentration of 5-HT failed to elicit any response, indicating that complete desensitization had occurred. If submaximal concentrations were used, a second response was observed, although it was smaller than the first, indicating partial desensitization. Replacing the bathing solutions following application of a maximal 5-HT concentration also reduced, but did not abolish, the degree of desensitization observed with a second application of 5-HT. In an in-vivo preparation, however, there was no diminution of the responses to four successive maximal doses of 5-HT. This lack of desensitization was also evident in the cardiovascular responses to 5-HT. It is concluded that desensitization to 5-HT is a phenomenon that is readily observed only in-vitro and which is probably related to the inability of a small amount of isolated tissue to eliminate 5-HT.

5-Hydroxytryptamine (5-HT) is present in abundance in the intestinal tract where it induces net fluid and electrolyte secretion and stimulates motility (Ormsbee & Fondacaro 1985). A well-established feature of 5-HT action in the intestine is agonist-induced desensitization of 5-HT receptors, demonstrated by the failure of repeated applications of 5-HT to elicit a response, a phenomenon that has been observed in both transport (Hubel 1984; Cooke & Carey 1985; Castro et al 1987) and motility (Craig et al 1990) studies. It also occurs in the rapidly-activating depolarization induced by 5-HT action on submucosal neurons (Cooke et al 1991; Frieling et al 1991). Since desensitization is receptor specific it can be used as a means of identifying 5-HT involvement in a particular response.

All the reports of 5-HT-receptor desensitization are based on studies performed in-vitro and it is not clear whether this phenomenon also occurs in-vivo. The present investigation was therefore designed to address this question using the Cl^- secretory response, measured electrically, as an index of one type of 5-HT action in the intestine.

Materials and Methods

Chemicals

Acetylcholine chloride and 5-hydroxytryptamine creatinine sulphate were obtained from Sigma Chemical Company Ltd, Poole, UK.

Animals

Experiments were carried out on male Wistar rats, 230–250 g, obtained from the Sheffield Field Laboratories and allowed free access to food and water. They were anaesthetized with sodium pentobarbitone (Sagatal, 60 mg kg^{-1}, i.p.).

Measurement of transintestinal electrical activity in-vitro

The potential difference (PD), short-circuit current (SCC) and resistance were measured in stripped sheets of intestine from which the outer muscle layers and myenteric plexus had been removed. Intestinal sheets, prepared from the proximal jejunum and distal ileum, were mounted in Ussing chambers with an aperture of 1·925 cm^2 and incubated at 37°C in Krebs bicarbonate saline gassed with 95% O_2–5% CO_2. The serosal solution contained 10 mM glucose and the mucosal solution 10 mM mannitol and each had a volume of 8 mL. The PD was measured using salt bridge electrodes connected via calomel half-cells to a differential input electrometer with output to a Vitatron 2-channel chart recorder (MSE Scientific Instruments, 2001 series). Current was applied across the tissue using conductive plastic electrodes and tissue resistance determined from the PD change induced by a 100 μA current pulse, taking the fluid resistance into account. The SCC generated by the tissue was calculated from PD and resistance measurements using Ohm's law.

Paired sheets of proximal jejunum and distal ileum, whose resistances did not vary by more than 25%, were used. After mounting they were allowed to stabilize for 10 min and then readings of basal electrical activity were taken for 10 min at 1-min intervals. 5-HT was added to the serosal solution of the test sheet, while the control sheet received an equivalent volume (100 μL) of the saline vehicle. After 10 min, 5-HT was added to both sheets. At the end of these experiments acetylcholine (10^{-3} M) was added to the serosal solution of both sheets to demonstrate the continued ability of the tissue to exhibit a secretory response.

The effects of replacing the incubation medium between 5-HT (10^{-4} M) applications were determined by removing both mucosal and serosal solutions 5 min after the first addition of 5-HT and making the second addition after a further 20 min. To test whether 5-HT remained in the bathing solution, the fluid removed from control and test sheets was added to separate sheets from the same animal that had not previously received any stimulus, and 5-HT (10^{-4} M) was added 10 min later.

To assess the concentration-dependence of 5-HT action,

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cumulative dose-response curves were constructed in which the next application of 5-HT was made at the peak of the response to the previous application as described by Bunce et al (1991).

Measurement of transintestinal electrical activity in-vivo

Segments of proximal jejunum and distal ileum, approximately 5 cm long, were isolated by tying off at the distal end and inserting a cannula into the proximal end. Both loops were then filled with 0·5 mL 154 mM NaCl. The PD across each loop was measured between a salt bridge electrode in contact with the luminal fluid and a common reference electrode in contact with the peritoneal fluid by means of a wick electrode. Each pair of electrodes was connected via calomel half-cells to a differential input electrometer. 5-HT was administered via a cannula in the femoral vein and each dose washed in with 0·2 mL 154 mM NaCl. Blood pressure was monitored at the femoral artery by a Druck pressure transducer (type 1175-L05) connected to a Lectromed chart recorder (Multitrace 4) and heart rate calculated from the pulse pressure by a Lectromed rate meter (model 5250). PD, blood pressure and heart rate were displayed on a computer using CED chart software and data were analysed by CED spike2 software. Rectal temperature was maintained at 37°C by a homeothermic blanket system (Harvard Apparatus Ltd, model 50-7061).

Expression of results

Results are expressed as mean values ± s.e.m. of the mean of the number of observations indicated. The basal electrical activity of all the sheets used is given, together with the number of animals from which they were taken. For each experimental group the values obtained were derived from tissues obtained from separate animals. A paired *t*-test was used to assess the significance of the difference between the responses to consecutive 5-HT applications. EC50 values are expressed as geometric means (95% confidence limits).

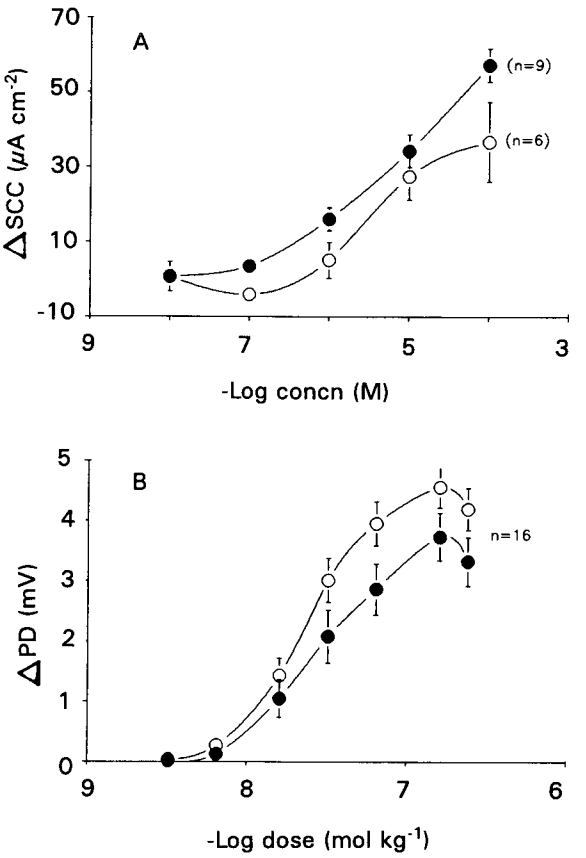

FIG. 1. Dose-dependence of 5-HT action on rat jejunum (○) and ileum (●) in-vitro (A) and in-vivo (B). The secretory response to 5-HT (applied serosally) was measured in-vitro as the rise in SCC (ΔSCC) generated by stripped intestinal sheets, and the dose-response curve determined cumulatively. In-vivo the response to 5-HT (administered intravenously) was monitored as an increase in PD (ΔPD), with 5-HT added sequentially. Each value represents the mean ± s.e.m. of the number of observations indicated.

Results

Response to 5-HT in-vitro

The jejunal (16 sheets from 11 animals) and ileal (83 sheets from 40 animals) sheets generated a basal PD of 2·2 ± 0·1 and 2·2 ± 0·1 mV, a SCC of 72 ± 4 and 46 ± 2 μA cm^{-2}, and had tissue resistances of 30 ± 1 and 49 ± 2 ohm cm^2, respectively. 5-HT induced concentration-dependent rises in the SCC generated by both jejunal and ileal sheets with maximum changes of 37 ± 11 (n = 6) and 57 ± 5 (n = 9) μA cm^{-2} and EC50 values of $1{\cdot}4 \times 10^{-5}$ ($2{\cdot}0 \times 10^{-6}$–$1{\cdot}1 \times 10^{-4}$) and $5{\cdot}0 \times 10^{-6}$ ($2{\cdot}0 \times 10^{-6}$–$1{\cdot}2 \times 10^{-5}$) M, respectively (Fig. 1).

The first application of a maximal concentration of 5-HT (10^{-4} M) induced a SCC response of 51 ± 20 (n = 5) μA cm^{-2} in the jejunum and 76 ± 5 (n = 25) μA cm^{-2} in the ileum. In the ileum this value was significantly greater ($P < 0{\cdot}05$) than the maximum response obtained from the cumulative dose-response curve, an observation that has also been reported in rat colon (Bunce et al 1991). A second application of the same concentration of 5-HT was, however, without significant effect (Table 1). When two consecutive lower concentrations (10^{-6} M) of 5-HT were added to previously untreated preparations of ileum, a second rise in SCC was obtained, although it was significantly lower ($P < 0{\cdot}01$) than the initial response (Table 1). Data from control sheets indicated that the lack of effect of the second application of 5-HT was not due to its later time of addition, as there was no significant difference ($P > 0{\cdot}05$ in all cases) between the responses of the control sheet and that of the test sheet to the first 5-HT challenge (Table 1).

The possibility that the lack of effect of a second application of a maximal concentration of 5-HT might be due to a failure in the responsiveness of the secretory process itself was tested by adding the secretagogue acetycholine at the end of the experiment. This induced a rise in SCC, the magnitude of which did not differ significantly ($P > 0{\cdot}05$) between the two sheets (Table 1).

The desensitization observed could be related to the continued presence of 5-HT in contact with the tissue and this was explored in the ileum by replacing the mucosal and serosal solutions between the first and second applications of a maximal concentration of the amine. This reduced the

Table 1. Change in SCC generated by stripped sheets of rat jejunum and ileum in response to two successive applications (applications 1 and 2) of 5-HT.

Application	1 Saline/5-HT	2 5-HT/5-HT	3 Acetylcholine/ acetylcholine
Jejunum			
10^{-4} M 5-HT (n = 5)			
Control	−7 ± 3	43 ± 8	219 ± 35
Test	51 ± 20	−3 ± 3	256 ± 38
P	< 0·05	< 0·01	> 0·05
Ileum			
10^{-4} M 5-HT (n = 5)			
Control	2 ± 1	78 ± 9	165 ± 8
Test	95 ± 5	0 ± 1	196 ± 16
P	< 0·001	< 0·001	> 0·05
10^{-6} M 5-HT (n = 6)			
Control	1 ± 1	21 ± 4	206 ± 23
Test	22 ± 5	7 ± 2	212 ± 15
P	< 0·01	< 0·01	> 0·05

5-HT was added to the serosal solution of test sheets at the concentrations indicated, the second application being made 10 min after the first. In control sheets the first application was an equivalent volume of the vehicle (100 μL 154 mM NaCl). Acetylcholine (10^{-3} M, application 3) was added to the serosal solution of all sheets 10 min after the second 5-HT application. The change in SCC is expressed as mean values in μA cm^{-2} ± s.e.m. of the number of observations indicated and a paired *t*-test was used to compare control and test sheets.

Table 2. Effect of replacing mucosal and serosal solutions on the desensitization of the response of stripped sheets of rat ileum to a second serosal application of 5-HT (10^{-4} M).

Application	1 Saline/5-HT	2 5-HT/5-HT
Control	1 ± 1	69 ± 12
Test	52 ± 7	20 ± 7
P	< 0·001	< 0·01

Mucosal and serosal solutions were replaced 5 min after the first addition of 5-HT (application 1) and the second addition of 5-HT (application 2) was made 20 min later. In control sheets the first application was an equivalent volume of vehicle (100 μL 154 mM NaCl). The change in SCC is expressed as mean values in μA cm^{-2} ± s.e.m. of six pairs of tissues and a paired *t*-test was used to compare control and test sheets.

degree of desensitization observed, as the second 5-HT challenge now induced a rise in SCC, although this was still significantly smaller ($P < 0.01$) than that obtained with the first addition of the amine (Table 2). The increased time interval between the two successive 5-HT applications in these experiments (25 min as opposed to 10 min in Table 1) was not responsible for the reduced desensitization, as a second dose of 5-HT (10^{-4} M) added 25 min after the first, with no replacement of the bathing solutions, still failed to increase the SCC (change in SCC = −1 ± 1 (n = 4) μA cm^{-2}, $P > 0.05$), although a response was obtained in control sheets (88 ± 25 (n = 4) μA cm^{-2}, $P < 0.05$). If the bathing solutions were removed from a preparation after the initial 5-HT response (91 ± 12 (n = 6) μA cm^{-2}) and then applied to a separate sheet, an increase in SCC of 83 ± 12 (n = 6) μA cm^{-2} was observed which was not significantly different from the response of the first sheet ($P > 0.05$). This could be attributed to the continued presence of the amine as the subsequent addition of 10^{-4} M 5-HT failed to elicit a significant rise in SCC (change in SCC = −2 ± 2 (n = 6) μA cm^{-2}, $P > 0.05$). Application to a separate preparation of the solutions bathing the control sheet (response to saline = −1 ± 1 (n = 6) μA cm^{-2}) did not increase the SCC (change in SCC = 6 ± 9 (n = 6) μA cm^{-2}, $P > 0.05$), but 10^{-4} M 5-HT added subsequently caused a rise of 85 ± 7 (n = 6) μA cm^{-2} which did not differ from the response of the test sheet ($P > 0.05$).

Application of 5-hydroxyindoleacetic acid, the major metabolite of 5-HT (Racké & Schwörer 1991), to the serosal solution at a concentration of 10^{-4} M did not cause a significant increase in the SCC generated by ileal sheets (change in SCC = −1 ± 1 (n = 4) μA cm^{-2}, $P > 0.05$). Addition of 5-HT (10^{-4} M) 10 min later, elicited a response of 80 ± 6 (n = 4) μA cm^{-2}, a value that did not differ significantly from the rise in SCC obtained with the same concentration of 5-HT in control sheets (72 ± 13 (n = 4) μA cm^{-2}, $P > 0.05$) where 5-hydroxyindoleacetic acid was not present.

Response to 5-HT in-vivo

The jejunum and ileum generated basal PD values of 5·2 ± 0·4 (n = 6) and 2·9 ± 0·7 (n = 6) mV, respectively, the serosal side being positive with respect to the mucosal side. 5-HT induced a dose-dependent rise in PD in both regions (Fig. 1). Administration of a maximal dose (1.6×10^{-7} mol kg^{-1}) of 5-HT increased the jejunal PD by 4·1 ± 0·3 (n = 6) mV and the ileal PD by 3·4 ± 0·6 (n = 6) mV. Three subsequent applications of 5-HT induced responses that did not differ significantly ($P > 0.05$ in all cases) from the first (Table 3).

As well as its effects on the intestine, intravenously administered 5-HT also affects the cardiovascular system (Kalkman et al 1984), causing an initial transient bradycardia mediated by 5-HT_3 receptors followed by a rise in blood pressure mediated by 5-HT_2 receptors and finally a sustained fall in blood pressure mediated by 5-HT_1-like receptors. These responses to 5-HT were not diminished on successive administration of maximal doses of the amine (Table 3).

Discussion

The increased electrical activity of the intestine that occurs in response to 5-HT challenge is a reflection of its ability to stimulate Cl^- secretion (Hardcastle et al 1981). This effect is observed both in-vivo and in-vitro (Fig. 1), but there appear to be differences in the behaviour of the two types of preparation in that desensitization of the response is observed in-vitro (Tables 1, 2), but not in-vivo (Table 3). The lack of desensitization in-vivo is not confined to the intestinal response, since the cardiovascular effects of successive applications of 5-HT were not diminished (Table 3).

The failure to obtain a second response to 5-HT in-vitro cannot be attributed to an inability of the enterocyte to secrete Cl^-. Acetylcholine stimulates intestinal secretion, activating the enterocyte by elevating intracellular Ca^{2+} (Hardcastle et al 1984), which is also considered to be involved in the secretory response to 5-HT (Donowitz et al 1980). When acetylcholine was added after 5-HT, it was able to elicit a rise in SCC indicative of the continued ability of the

Table 3. Effect of four successive applications of 5-HT ($1{\cdot}6 \times 10^{-7}$ mol kg^{-1}, i.v.) at 8-min intervals.

5-HT dose	Increase in PD (mV)		Decrease in heart rate (beats min^{-1})	Change in blood pressure (mm Hg)	
	Jejunum	Ileum		Systolic	Diastolic
1	$4{\cdot}1 \pm 0{\cdot}3$	$3{\cdot}4 \pm 0{\cdot}6$	54 ± 15	$+23 \pm 5$	-58 ± 6
2	$3{\cdot}9 \pm 0{\cdot}4$	$3{\cdot}1 \pm 0{\cdot}5$	53 ± 14	$+16 \pm 2$	-59 ± 5
3	$3{\cdot}1 \pm 0{\cdot}4$	$2{\cdot}9 \pm 0{\cdot}6$	49 ± 13	$+13 \pm 2$	-58 ± 4
4	$3{\cdot}4 \pm 0{\cdot}4$	$2{\cdot}9 \pm 0{\cdot}7$	49 ± 12	$+11 \pm 2$*	-54 ± 5

Basal values for cardiovascular function were heart rate $= 462 \pm 12$ beats min^{-1}, systolic blood pressure $= 158 \pm 4$ mmHg and diastolic blood pressure $= 130 \pm 6$ mmHg. Results are expressed as mean values $\pm$ s.e.m. of six animals. *$P < 0{\cdot}05$ compared with the previous dose values.

enterocyte to exhibit a secretory response. Desensitization cannot therefore be attributed to a loss of responsiveness of the secretory process or the intracellular signalling system that leads to its activation. Similar conclusions have been drawn from studies of bethanechol-induced intestinal secretion, where desensitization to the cholinergic agonist is not followed by a failure of the response to prostaglandin E_2 (Przyborski et al 1991). This phenomenon is likely to occur at the receptor level, but the failure to observe this behaviour in-vivo requires explanation. The continued presence of 5-HT in contact with its receptor sites may be an important factor, since replacement of 5-HT-containing bathing solutions before the second addition of the amine reduced the degree of desensitization that occurred (Table 2). The continued presence of the amine in the solution bathing the desensitized preparation is confirmed by the rise in SCC that occurs when this solution is transferred to a sheet that has not previously been exposed to 5-HT. The fact that this sheet then became desensitized to subsequent stimulation by 5-HT indicates that the active component in the transferred solution was 5-HT.

The possibility that desensitization could be associated with the accumulation of the product of 5-HT degradation was tested by examining the effects of 5-hydroxyindoleacetic acid, the major metabolite of 5-HT in the intestine (Racké & Schwörer 1991). This agent had no effect on the electrical activity of intestinal sheets, confirming previous in-vivo data (Hardcastle et al 1981). Moreover, prior application of 5-hydroxyindoleacetic acid did not alter the response to subsequently administered 5-HT, indicating that desensitization could not be attributed to accumulation of this metabolite.

In-vivo, 5-HT is removed both by the circulation and the catabolic processes that exist in most tissues of the body. The amount of 5-HT required to elicit a maximal response in-vitro (5×10^{-7} mol) exceeds that required in-vivo ($3{\cdot}3 \times 10^{-8}$ mol). Thus, a much greater quantity of 5-HT has to be removed in-vitro, in spite of the fact that the amount of tissue available for catalysis is much less.

It therefore appears that desensitization of the 5-HT receptors that stimulate intestinal secretion is a phenomenon that is readily detected only in-vitro, arising, at least in part, from the inability of the relatively small amount of isolated tissue to inactivate the secretagogue.

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