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Involvement of nitric oxide in the response to 5-hydroxytryptamine in the rat in-vivo

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Abstract—The involvement of nitric oxide (NO) in the effects of 5-HT on intestinal secretion and cardiovascular function in anaesthetized rats was investigated using N^G-nitro-L-arginine methyl ester (L-NAME), a specific NO-synthase antagonist, and its optical isomer D-NAME. L-NAME significantly reduced the prolonged hypotensive response to 5-HT. It also caused a small rightward shift in the colonic 5-HT dose-response curve. This suggests that NO plays a significant role in the prolonged hypotensive response to 5-HT, and may make a small contribution to the secretory response of the colon, but not that of the jejunum, in the rat in-vivo.

5-Hydroxytryptamine (5-HT) is known to induce intestinal secretion (Hardcastle et al 1981), but the mechanisms responsible remain unclear, with evidence for both a direct action of 5-HT on the enterocyte (Hirose & Chang 1988) and indirect effects mediated by the enteric nervous system (Franks et al 1993). It has recently been reported that 5-HT induces a relaxation of gastrointestinal smooth muscle which is mediated by nitric oxide (NO) (Bogers et al 1991; Allescher et al 1992), an agent that has now been implicated in a range of physiological processes throughout the body (see Moncada et al (1991) for review).

The involvement of NO in 5-HT-induced intestinal secretion was investigated in-vivo using the specific inhibitor of NO synthase, N^G-nitro-L-arginine methyl ester (L-NAME) (Moncada et al 1991). The preparation used provided an opportunity to examine not only the intestinal effects of 5-HT, but also the changes in cardiovascular function that follow intravenous administration of the amine.

Materials and methods

Male Wistar rats, 230–250 g, from the Sheffield Field Laboratories, with free access to food and water, were anaesthetized by intraperitoneal injection of 70 mg kg^{-1} sodium pentobarbitone. Following tracheotomy, 5 cm segments of proximal jejunum and colon were isolated by tying off at the distal end and inserting a cannula into the proximal end. The contents were washed out and the loops filled with warm 154 mM NaCl. The potential difference (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. Blood pressure was measured via the femoral artery using a Druck pressure transducer (type 3389). Heart rate was calculated from the pulse pressure by a Lectromed rate meter (model 5250). Jejunal and colonic PD values, blood pressure and heart rate were all recorded on computer using CED Chart software. All drugs were administered via a cannula in the femoral vein.

Non-cumulative dose-response curves to 5-HT were constructed in the absence and presence of either L-NAME or D-NAME (1·3 followed by 13 μmol kg^{-1}). Data were analysed using CED Spike2 software and Student's unpaired t-test (except where otherwise stated) was used for statistical analysis.

Acetylcholine chloride, 5-hydroxytryptamine creatinine sulphate (5-HT), N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-D-arginine methyl ester (D-NAME) and atropine methyl nitrate were obtained from Sigma Chemical Co., Poole, UK.

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Results

Intestinal responses. Basal PD values in the jejunum and colon were 6·4 ± 0·3 and 14·5 ± 0·6 mV, respectively (n = 10), the serosa being positive with respect to the mucosa.

5-HT induced a dose-dependent rise in transintestinal PD in both the jejunum and the colon with maximum responses of 4·4 ± 0·3 and 7·9 ± 0·7 mV, and EC50 values of 27 ± 2·3 and 42 ± 5·6 nmol kg^{-1}, respectively (n = 10). These values were not significantly affected by D-NAME (Table 1). L-NAME (neither 1·3 nor 13 μmol kg^{-1}) had no effect on the 5-HT-induced maximum rise in transintestinal PD (PD_{max}) in either of the regions investigated when compared with values obtained in the presence of equimolar D-NAME. It did, however, increase the colonic EC50 value from 42 ± 5 to 69 ± 10 nmol kg^{-1} with 1·3 μmol kg^{-1} and to 87 ± 20 nmol kg^{-1} with 13 μmol kg^{-1} ($P < 0·05$

Table 1. Effect of 1·3 and 13 μmol kg^{-1} D-NAME and L-NAME on the 5-HT-induced rise in transmural PD in the jejunum and colon. 5-HT action is expressed in terms of the maximum increase in PD (PD_{max}) and the EC50 value.

	Control (10)	D-NAME (μmol kg^{-1}) (5)		L-NAME (μmol kg^{-1}) (5)	
		1·3	13	1·3	13
PD_{max} (mV)					
Jejunum	4·4±0·3	5·1±0·4	5·1±0·5	4·3±0·4	4·1±0·5
Colon	7·9±0·7	8·4±1·0	8·5±1·0	8·9±1·2	8·8±0·9
EC50 (nmol kg^{-1})					
Jejunum	27±2·3	28±2·9	36±6·4	34±3·5	37±4·9
Colon	42±5·6	57±8·4	60±5·1	69±10·0*	87±20·0*

Each value represents the mean±s.e.m. of the number of observations indicated, and an unpaired t-test was used to assess the significance of D-NAME and L-NAME action, *$P<0·05$.

in both cases, Table 1). The EC50 value for the jejunum was unaffected.

Cardiovascular responses. The higher dose of L-NAME increased basal systolic blood pressure (BP_s) from 160±4 (n=10) to 183±3 mmHg (n=5, $P<0·01$) and diastolic blood pressure (BP_d) from 113±2 (n=10) to 131±2 mmHg (n=5, $P<0·001$). These values were significantly greater than those obtained with the same dose of D-NAME ($P<0·01$ and $P<0·001$, respectively, n=5).

Intravenous administration of 5-HT induces a triphasic cardiovascular response, with each phase being mediated via a different 5-HT-receptor subtype (Kalkman et al 1984). The initial phase is a transient fall in blood pressure and heart rate known as the Bezold-Jarisch reflex. The second phase is a brief pressor response followed by the third phase, a prolonged hypotension (Fig. 1). Each of these responses to 5-HT is dose-dependent (Fig. 2). Neither the maximum fall in heart rate (246±14 beats min^{-1}, n=10) nor the maximum rise in BP_s (37±5 mmHg, n=10), was altered by either L-NAME or D-NAME ($P>0·05$, paired t-test in both cases). Unlike the intestinal response, the Bezold-Jarisch reflex and the pressor phase, the prolonged hypotension exhibits desensitization to 5-HT on the third (but not second) dose-response curve, the maximum fall in BP_d being 18% less than that induced by the earlier applications of 5-HT ($P<0·001$ paired t-test). Neither 1·3 μmol kg^{-1} L-NAME nor the same dose of D-NAME influenced this hypotensive phase. The higher dose of L-NAME reduced the 5-HT-induced hypotensive phase, causing a 41·6±4·1% inhibition of the maximum response (Fig. 3), which was significantly greater than that observed with equimolar D-NAME

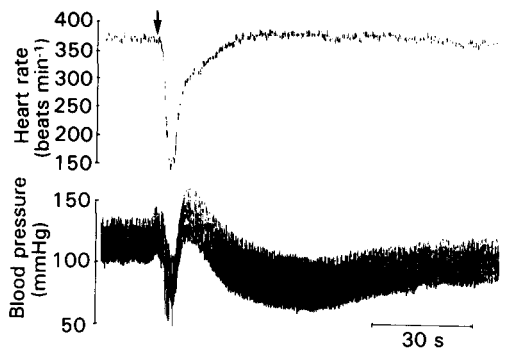

FIG. 1. Typical cardiovascular response to intravenous administration of 240 nmol kg^{-1} 5-HT at the point indicated.

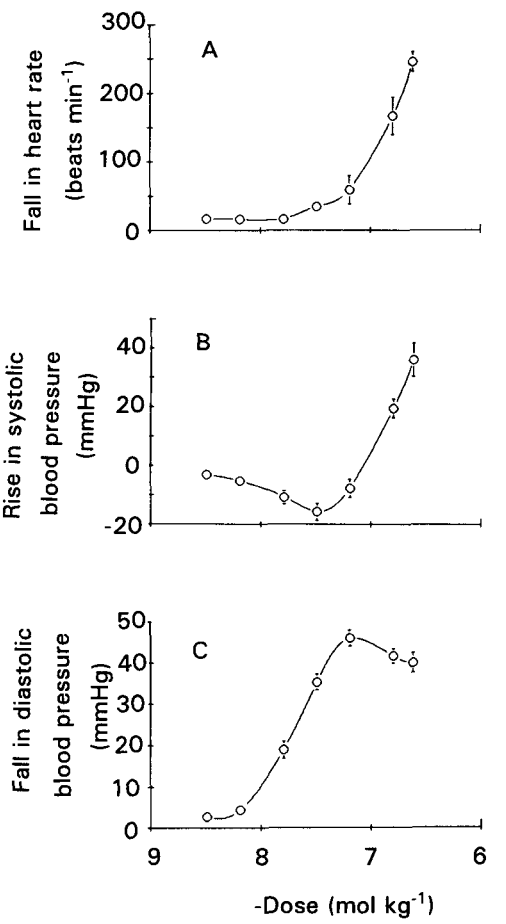

FIG. 2. Dose-dependence of the triphasic cardiovascular response to 5-HT. The fall in heart rate (A), the rise in systolic blood pressure (B) and the prolonged fall in diastolic blood pressure (C) are plotted as a function of log 5-HT dose. Each point represents the mean±s.e.m. of 10 observations.

($P<0·001$). This, in turn, was no larger than that obtained in a third successive control 5-HT dose-response curve.

Atropine (3·4 μmol kg^{-1}) abolished the fall in blood pressure induced by 14 μmol kg^{-1} acetylcholine (control 86±1·5 mmHg; +atropine 3±0·6 mmHg, $P<0·001$, n=6). This dose of atropine, however, had no effect on the prolonged fall in blood pressure induced by 5-HT ($P>0·05$ paired t-test, n=6).

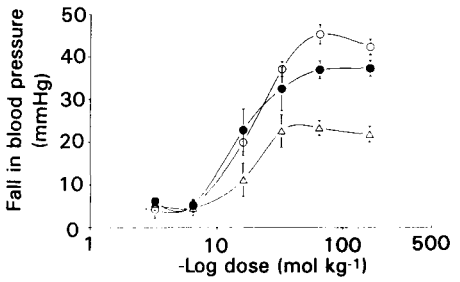

FIG. 3. Effect of L-NAME on the prolonged hypotensive phase induced by 5-HT. The fall in blood pressure induced by 5-HT alone (○) and in the presence of 1·3 (●) and 13 μmol kg^{-1} (△) L-NAME is plotted as a function of log 5-HT dose. Each point represents the mean ± s.e.m. of five observations.

Discussion

The triphasic cardiovascular response to 5-HT is mediated via three different 5-HT receptors. The first phase, the Bezold-Jarisch reflex, is initiated by 5-HT$_3$ receptors on afferent vagal nerves fibres at the level of the right ventricle. The second pressor phase is mediated by 5-HT$_2$ receptors, and the third hypotensive phase by 5-HT$_1$-like receptors, both presumably on the vascular smooth muscle cells (Kalkman et al 1984).

The stereospecific inhibition of the hypotensive phase by L-NAME suggests that activation of the 5-HT$_1$-like receptor leads to release of NO. Indeed, Allescher et al (1992) demonstrated that 5-HT$_1$ receptor-mediated relaxation in the guinea-pig colon involved NO release. The hypotensive response of anaesthetized rats to acetylcholine has also been shown to involve the release of NO (Rees et al 1989). However, atropine at a dose that abolishes the acetylcholine-induced fall in blood pressure in-vivo, had no effect on the 5-HT-induced hypotensive phase, indicating that acetylcholine does not act as an intermediary. The NO released on 5-HT$_1$-receptor activation may cause a direct relaxation of the blood vessels, the resultant vasodilatation being responsible for the prolonged hypotensive response to 5-HT stimulation.

At the higher dose, L-NAME caused a rise in blood pressure, suggesting that NO is released endogenously to create a vasodilatory tone. This confirms earlier observations using the NO-synthase inhibitor L-NMMA (Rees et al 1989).

In addition to its cardiovascular actions, NO has been shown to be involved in some gastrointestinal functions e.g. castor oil-induced fluid and Na^+ secretion (Mascolo et al 1993) and hormonally-stimulated pancreatic secretion (Konturek et al 1993). In the pancreas, NO probably enhances secretion by changing the perfusion of the vascular bed as NO-synthase inhibitors reduce pancreatic blood flow.

Throughout the intestinal tract, 5-HT induces a dose-dependent increase in transmural PD which is thought to reflect electrogenic Cl^- secretion (Hardcastle et al 1981). L-NAME had no effect on either the PD_{max} or EC50 values for 5-HT in the jejunum. In the colon, however, L-NAME increased the EC50 value for 5-HT without altering the PD_{max}, suggesting that NO might contribute to the secretory response in this region of the gut.

It is concluded that NO plays a significant role in the prolonged hypotensive phase of the cardiovascular response to 5-HT. It may also make a small contribution to the secretory response of the colon, but not that of the jejunum, in the rat in-vivo.

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