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Ketanserin potentiates the prejunctional inhibitory effect of 5-hydroxytryptamine on rat vas deferens

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5-Hydroxytryptamine (5-HT) slightly inhibited the twitch contractions of rat vas deferens caused by single pulse field stimulation at 0·1 Hz. The inhibitory effect of 5-HT was much less in the epididymal portion than in the prostatic portion of the vas deferens. Ketanserin potentiated the prejunctional inhibitory effect of 5-HT and attenuated its stimulatory effect. This potentiation was observable only in the epididymal portion, of the vas deferens. Cyproheptadine and mianserin, but not methysergide, had essentially similar potentiating effects to those of ketanserin. These results suggest that the 5-HT receptor that mediates prejunctional inhibition is not of the 5-HT_2 type, and that ketanserin acts by suppressing the 5-HT-induced stimulatory effect, which is possibly mediated by a postjunctional 5-HT_2 receptor, thus unmasking the inhibitory effect of 5-HT.

A prejunctional inhibitory action of 5-hydroxytryptamine (5-HT) on adrenergic transmission has been demonstrated in the heart (Martinez & Lokhandwala 1980), and in other vascular preparations, such as the saphenous vein (McGrath 1977; Feniuk et al 1979; Watts et al 1981), basilar artery (Bevan et al 1975) and mesenteric artery (Su & Uruno 1985). Moreover, 5-HT was reported to inhibit stimulation-induced contractions of rat vas deferens via a prejunctional 5-HT receptor (Kapur & Mottram 1979). However, in many preparations, we failed to demonstrate 5-HT-induced inhibition of twitch contractions; we found that 5-HT had a variable effect, and in some preparations actually increased the twitch response and the basal tone. In rat mesenteric artery, concentrations of 5-HT that alone caused vasoconstriction were reported to produce vasodilation in the presence of ketanserin (McLennan & Taylor 1984). Therefore, we examined whether ketanserin unmasks 5-HT-induced inhibition of the twitch response of rat vas deferens by suppressing the stimulatory effect of 5-HT. In addition, since two distinct types of excitatory innervation, adrenergic and non-adrenergic, have been demonstrated in the rat vas deferens, each of which is predominant in one end of the preparation (McGrath 1978), we also examined whether the inhibitory effect of 5-HT varied in different portions of the vas deferens.

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MATERIALS AND METHODS

Male Wistar rats aged 8 to 9 weeks (about 200 g) were used. The vas deferens was excised and separated from connective tissue under a dissecting microscope. In some experiments, the vas deferens was cut into epididymal and prostatic portions constituting 60 and 40%, respectively, of its total length. The preparation was set up in a 10 ml organ bath containing Krebs solution of composition (mM): NaCl 115·3, KCl 4·7, $CaCl_2$ 1·6, $MgSO_4$ 1·2, $NaHCO_3$ 25·0, KH_2PO_4 1·2 and glucose 11·1. The medium was maintained at 32 °C and bubbled with 5% CO_2 in O_2. The resting tension was maintained at 200 mg. After equilibration of the preparation for 60 min, field stimulation of 0·3 ms duration was applied at 0·1 Hz from a stimulator (Nihon Kohoden SEN 3201) with a constant voltage modulator through a pair of platinum electrodes, one attached to one end of the preparation, and the other (reference) placed around it. Contractions were recorded isometrically with a force displacement transducer (Nihon Kohoden SD-1TH). The twitch contractions elicited by field stimulation were abolished by either tetrodotoxin (10^{-7} M) or guanethidine (3×10^{-6} M), indicating that the response was neurogenic. For construction of a dose-response curve, 5-HT was

added cumulatively in a volume of 10 to 70 μl to the 10 ml organ bath. Ketanserin and other inhibitors were applied 10 min before 5-HT, unless otherwise stated. pD_2 and pA_2 values were calculated by the method of Van Rossum (1963). As complete dose-response curves for 5-HT were not constructed, the inhibitory potencies of 5-HT in the absence and presence of ketanserin were expressed as apparent pD_2 values, calculated from the IC50 values, which were tentatively estimated taking the respective inhibitions by 10^{-5} M 5-HT as 100%. The significance of difference between values was examined by the paired *t*-test, and a *P* value of less than 0·05 was considered significant.

The drugs used were 5-hydroxytryptamine creatinine sulphate (5-HT, Sigma Chemical Co., St Louis MO), noradrenaline bitartrate (Sigma), methysergide (Sandoz, Switzerland), cyproheptadine hydrochloride (Merck Sharp and Dohme Research Laboratories, West Point, PA), mianserin hydrochloride (Research Biochemicals Inc., Wayland, MA), ketanserin (Janssen Pharmaceutica, Beerse, Belgium), prazosin (Taito-Pfeizer, Tokyo, Japan) and desipramine hydrochloride (Fujisawa, Osaka, Japan).

RESULTS

Effect of 5-HT on the twitch contraction

Fig. 1 shows a selected tracing from a record demonstrating the typical response of rat vas deferens to 5-HT added cumulatively in the absence and presence of ketanserin. 5-HT at concentrations above 10^{-8} M inhibited the twitch contractions of the tissue caused by single pulse field stimulation at 0·1 Hz. Although 5-HT at above 3×10^{-7} M caused transient, slight increase in the basal tone, it inhibited the twitch response further. Dose-response curves for the inhibitory effect of 5-HT are shown in Fig. 2A. The maximal inhibition, attained at 10^{-5} M, was 43·3 ± 5·0% (n = 8) and the pD_2 value was 6·79 ± 0·16 (n = 8). 5-HT in the range causing inhibition (10^{-8} – 10^{-5} M) did not inhibit, rather it slightly augmented noradrenaline (NA)-induced contractions, which were the same as those induced by field stimulation only.

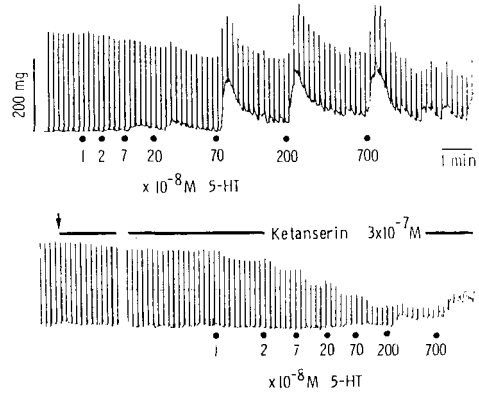

FIG. 1. Selected tracings from a record showing the response of rat vas deferens to single pulse field stimulation at 0·1 Hz. Ketanserin was added 10 min before 5-HT. 5-HT was added cumulatively at the times indicated by dots. The arrow and underline indicate the time of application and the presence of ketanserin, respectively.

Potentiation by ketanserin of the 5-HT-induced inhibition

Ketanserin at concentrations above 10^{-8} M augmented, rather than antagonized, the inhibitory effect of 5-HT, without appreciably affecting the threshold concentration of 5-HT for inhibition. In addition, ketanserin suppressed the increase in the basal tone induced by 5-HT. As shown in Figs 1 and 2A, pretreatment with 3×10^{-7} M ketanserin, besides causing 17·1 ± 4·1% (n = 8) decrease in the basal twitch contractions, increased the inhibition of the twitch response by 5-HT 10^{-7} M from 25·3 ± 3·8 to 51·1 ± 3·3% (n = 8, $P < 0·01$). Ketanserin shifted the dose-response curve for 5-HT downwards. Ketanserin at 3×10^{-7} M increased the inhibition by 5-HT 10^{-5} M from 43·3 ± 5·0 to 90·5 ± 4·8% (n = 8, $P < 0·01$), but did not affect the pD_2 value of 5-HT significantly [6·96 ± 0·12 with ketanserin vs 6·79 ± 0·16 without ketanserin (n = 8, $P < 0·05$)]. Even when 5-HT had no effect or slightly increased the twitch response, ketanserin unmasked its inhibitory effect.

The α_1-antagonist prazosin inhibited NA-induced contractions with a pA_2 value of 8·03 ± 0·06 (n = 6). But even at a concentration of 3×10^{-7} M which by itself reduced the twitch response by 30·6 ± 3·1% (n = 5), it did not potentiate or unmask the inhibitory effect of 5-HT.

Effects of ketanserin and prazosin on the 5-HT-induced contractions

Ketanserin, 10^{-7} M, which potentiated the 5-HT-induced inhibition, attenuated the contractile actions of 10^{-5} and 3×10^{-5} M 5-HT by 89·3 ± 6·1 and 67·7 ± 4·9% (n = 4), respectively. In the presence of 3×10^{-8} M ketanserin, the dose-response curve for 5-HT was shifted about 3 times to the right, the pA_2 value being 7·93 ± 0·18 (n = 4). However, with increase in the concentration of ketanserin to 10^{-7}, 3×10^{-7} and 10^{-6} M, further shifts of the curve were only slight,

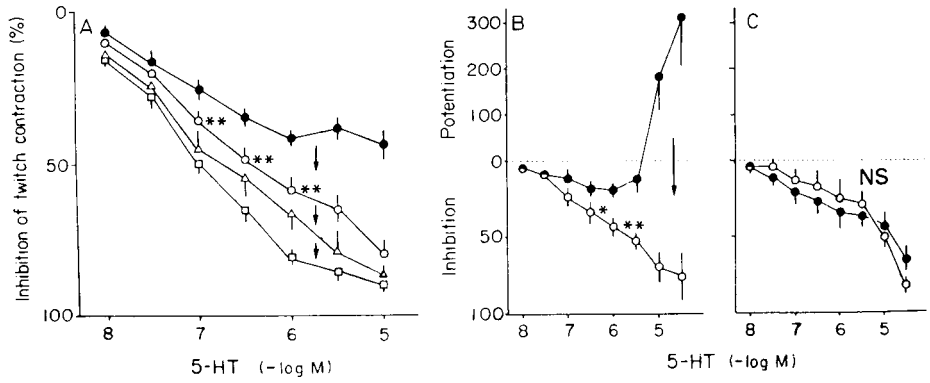

FIG. 2. Dose-response curves for the inhibitory effects of 5-HT on different portions of rat vas deferens in the absence and presence of ketanserin. A, whole vas deferens; B, epididymal portion of vas deferens; C, prostatic portion of vas deferens. ●, control: ○, with 10^{-8} M ketanserin (in B and C, with 3×10^{-7} M ketanserin); △, with 10^{-7} M ketanserin; □, with 3×10^{-7} M ketanserin. The ordinates indicate inhibition or potentiation of the twitch response, as percentages of the initial values just before addition of 5-HT in the absence or presence of ketanserin. Values are means ± s.e.m. of values for 8 (A) and 6 (B and C) preparations. NS, not significant. * $P < 0.05$, ** $P < 0.01$, compared with the respective control value (paired t-test).

and the pA_2 values decreased to 7·26 ± 0·20, 7·03 ± 0·11 and 6·12 ± 0·21 (n = 4), respectively. Ketanserin had less effect on the contractile action of NA, its pA_2 value against NA being 6·01 ± 0·12 (n = 5). The dose-response curve for 5-HT became biphasic in the presence of prazosin (data not shown); prazosin at concentrations of up to 3×10^{-7} M did not affect the contractions induced by low concentrations of 5-HT ($10^{-7} - 10^{-6}$ M), but antagonized those induced by high concentrations of 5-HT (above 3×10^{-6} M) with a pA_2 value of 8·29 ± 0·08 (n = 6).

Interaction of 5-HT with ketanserin on different portions of the vas deferens

Next, we examined the effects of 5-HT with and without ketanserin on the epididymal and prostatic portions of the vas deferens (Fig. 2, B and C). 5-HT had more inhibitory effect on the prostatic portion than on the epididymal portion. In the prostatic portion, the inhibition with 3×10^{-5} M 5-HT was 68·0 ± 10·8% (n = 6), and ketanserin, even at 3×10^{-7} M, did not increase this inhibition. On the other hand, in the epididymal portion, the maximal inhibition, attained with 10^{-6} M 5-HT, was only 21·6 ± 5·3% (n = 6). The inhibition was reduced to 11·9 ± 6·1% with 5-HT at 3×10^{-6} M, and further increase in the concentration of 5-HT augmented the twitch contractions with transient increase in the basal tone: 5-HT at 3×10^{-5} M increased the twitch height 319·7 ± 102·4% (n = 6). Under these conditions, ketanserin at 3×10^{-7} M potentiated the inhibition caused by low concentrations of 5-HT as observed with whole vas deferens. Furthermore, ketanserin at 3×10^{-7} M reversed the enhancement of the twitch contractions produced by high concentrations of 5-HT to inhibition: it reversed the 319% enhancement by 3×10^{-5} M 5-HT to 75·1 ± 8·2% inhibition (Fig. 2B).

Effects of other 5-HT antagonists in the epididymal portion

The effects of cyproheptadine, mianserin and methysergide on the epididymal portion of the vas deferens were examined (Fig. 3). Cyproheptadine and mianserin ($10^{-7} - 3 \times 10^{-6}$ M) showed similar potentiating effects to that of ketanserin without antagonizing the inhibitory effect of 5-HT, but their potencies were less than that of ketanserin. Methysergide at 10^{-7} to 10^{-5} M tended to potentiate the 5-HT-induced inhibition of the twitch response, but its effect was not statistically significant (n = 6, $0.05 < P < 0.01$), and at above 3×10^{-5} M it antagonized the inhibitory action of 5-HT significantly. Mianserin (10^{-6} M), cyproheptadine (3×10^{-7} M) and methysergide (10^{-6} M) abolished the contractions induced by 10^{-5} M 5-HT.

DISCUSSION

Ketanserin, while attenuating the 5-HT-induced increase in the basal tone, potentiated the prejunctional inhibitory effect of 5-HT on the twitch response of rat vas deferens mainly in the epididymal portion where the adrenergic component is predominant (Anton et al 1977; McGrath 1978). Ketanserin may act by suppressing the postjunctional α_1-action

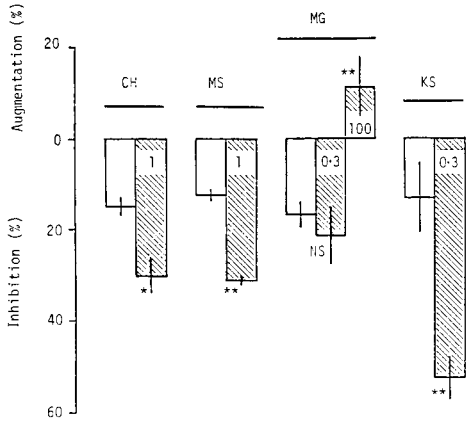

FIG. 3. Comparison of the effects of 5-HT antagonists on the 5-HT (3×10^{-6} M)-induced inhibition of the twitch contractions of the epididymal portion of rat vas deferens. CH, cyproheptadine; MS, mianserin; MG, methysergide; KS, ketanserin. Open columns, without antagonists; hatched columns, with antagonists. Concentrations of antagonists are indicated in μM in the columns. The ordinate shows inhibition (downwards) or augmentation (upwards) of the twitch contractions, taking the control amplitude of the twitch response as 100%. Values are means ± s.e.m. of values in 6 preparations. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, compared with the respective control value (paired t-test).

of 5-HT (Lucchelli et al 1984) or NA release from neuronal stores, which probably occurs by displacement (McGrath 1977; Hay & Wadsworth 1982), and which complicates the inhibitory effect of 5-HT. However, a possible α_1-blocking action of ketanserin does not seem to be important for its potentiating effect because (i) ketanserin at the concentrations necessary for potentiation (below 3×10^{-7} M) did not show any appreciable α_1-blocking action, (ii) prazosin did not affect the inhibitory effect of 5-HT, (iii) the uptake$_1$ blocker desipramine did not affect the inhibitory action of 5-HT, and (iv) the inhibitory action of 5-HT on the twitch response and direct contractile action were apparent at much lower concentrations than those reported to be necessary for release of NA by displacement (Humphrey 1978; Lucchelli et al 1984).

Alternatively, it seems possible that ketanserin potentiates the inhibitory effect of 5-HT by suppressing its postjunctional stimulatory effect, which could interfere with the inhibitory effect. The receptor mediating the stimulatory effect of 5-HT on the vas deferens may be of the 5-HT$_2$ type, because the contractile action of 5-HT was antagonized by ketanserin with a pA_2 value of 8·0, and because the vasoconstrictor action of 5-HT was reported to be blocked by ketanserin (Cohen et al 1981, 1983; Leysen et al 1982; Su & Uruno 1985; Van Nueten et al 1981). The present findings in rat vas deferens have some similarities to observations that 5-HT produced either vasoconstriction or vasodilation depending on the preparation and experimental conditions. In partially constricted rat mesenteric artery, 5-HT has been found to induce vasodilation or vasoconstriction, and ketanserin has been found to antagonize the 5-HT$_2$ receptor-mediated vasoconstriction and to potentiate the vasodilation (McLennan & Taylor 1984). Moreover, ketanserin has been found to enhance or unmask 5-HT-mediated vasodilation (Cocks & Angus 1983; Verdouw et al 1984) by inhibiting 5-HT$_2$-mediated vasoconstriction. A similar potentiating effect of cyproheptadine on the inhibition by 5-HT of electrically induced vasoconstriction has been attributed to its ability to inhibit the contractile action of 5-HT, although the receptor type for cyproheptadine was not defined (Watts et al 1981).

Cyproheptadine and mianserin were shown to have relatively high affinities for the 5-HT$_2$ receptor in rat cortex preparations (Awouters et al 1982). Therefore, these antagonists are thought to potentiate the action of 5-HT in a similar manner to that of ketanserin. In the present experiments we found that antagonists with high affinities for the 5-HT$_2$ receptor (Awouters et al 1982) were the more potent in augmenting 5-HT-induced inhibition, the order of their potencies being ketanserin > cyproheptadine = mianserin ≫ methysergide.

These findings raise the question of why methysergide, an antagonist of both 5-HT$_2$ and 5-HT$_1$ receptors (Awouters et al 1982) caused little potentiation, and at high concentrations antagonized the action of 5-HT. The partial agonist characteristics of methysergide (Apperley et al 1980) may be responsible for its inability to potentiate the action of 5-HT, because methysergide has been demonstrated to be an agonist in rabbit mesenteric artery (Moritoki & Su 1981), and dog saphenous vein (Watts et al 1981) and to act as an antagonist in rat vas deferens (Kapur & Mottram 1979). In addition, the possible 5-HT$_1$ antagonistic action of methysergide may also complicate its 5-HT$_2$ antagonistic action.

The 5-HT receptor mediating the prejunctional inhibition may be of the non-5-HT$_2$ type, because ketanserin, cyproheptadine, methysergide (except at high concentrations) and mianserin, which have been shown to be 5-HT$_2$ antagonists, did not antagonize the inhibitory action of 5-HT. The potencies of 5-HT agonists in inhibiting NA release from the saphenous vein were reported to be well

correlated with their binding affinities for the 5-HT_1 receptor in brain (Engel et al 1983). In addition, the prejunctional inhibitory 5-HT receptor mediating NA release from vascular adrenergic nerves (Cohen 1985) and acetylcholine release from ileal cholinergic nerves (Kilbinger & Pfeuffer-Friederich 1985) has been concluded to be of the 5-HT_1 type, because 5-HT antagonists with binding affinities for the 5-HT_1 site antagonized the 5-HT-induced inhibition of transmitter release. In the present experiments, relatively high concentrations of methysergide (above 10^{-5} M), which is known to have affinity for the 5-HT_1 site as well as the 5-HT_2 site (Leysen et al 1982; Martin & Sanders-Bush 1982), antagonized the inhibitory effect of 5-HT on the vas deferens. From this indirect evidence and also from the reported evidence, the inhibitory prejunctional receptor in rat vas deferens is assumed to be of the 5-HT_1 type.

The inhibitory effect of 5-HT on the rat vas deferens was variable. In some preparations, the twitch contractions were almost completely suppressed by 5-HT, whereas in others 5-HT enhanced, rather than inhibited the twitch response. Two distinct types of responses have been demonstrated: an adrenergic component in the epididymal portion, and a non-adrenergic component in the prostatic portion (McGrath 1978). This complex mechanism could be responsible for the variable effect of 5-HT. Indeed, in the present work we found that the inhibitory effect of 5-HT was greater in the prostatic portion than in the epididymal portion of the preparation, and that the effect in the prostatic portion was exclusively inhibitory. The variability in the response of whole vas deferens to 5-HT may be due to differences in the balance between the stimulatory and inhibitory response to 5-HT and also to the complex innervation.

On the basis of the present findings and the above considerations, we conclude that 5-HT has prejunctional inhibitory effect at low concentrations, and a postjunctional stimulatory effect at higher concentrations, and that ketanserin unmasks the prejunctional inhibitory effect of 5-HT, possibly by suppressing the stimulatory effect, which is mainly on the epididymal portion of the vas deferens.

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