Interaction of 5-Hydroxytryptamine and Ketanserin in Rat Vas Deferens Subjected to Low Frequency Field Stimulation

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Abstract—The interaction of 5-hydroxytryptamine (5HT) and ketanserin was investigated in isolated rat vas deferens. Ketanserin (10^{-7} M) almost completely abolished the phasic and the following rhythmic contractions induced by 5HT, whereas the inhibition by prazosin (10^{-6} M) or methysergide (10^{-6} M) of 5HT-induced contractions were incomplete. The amplitude of twitch contractions of vas deferens subjected to low frequency (0.1 Hz) field stimulation were substantially unchanged by 5HT (10^{-7}–10^{-5} M) per se. After pretreatment of the tissue with ketanserin (10^{-8}–10^{-6} M), 5HT, in a concentration-dependent manner, attenuated the amplitude of twitch contractions. Such attenuation of the amplitude was not observed after pretreatments with methysergide (10^{-8}–10^{-6} M) or prazosin (10^{-7}–10^{-5} M). The 5HT-induced inhibition of twitch contractions in the presence of ketanserin was not antagonized by phentolamine, propranolol, methysergide, morphine, promethazine, cimetidine, atropine or indomethacin. It is suggested that 5HT has dual (excitatory and inhibitory) effects upon nerve transmission of rat vas deferens, and only the excitatory effect is suppressed by ketanserin.

5-Hydroxytryptamine (5HT, serotonin) receptors are present on many types of neurons in the peripheral nervous system (1). Also, in vas deferens, some studies (2, 3) have suggested that 5HT might play a role in the regulation of nerve transmission. Ketanserin, a new quinazoline derivative, is reported to be a selective blocker of 5HT receptors found on vessel walls, bronchial muscles and platelets (5HT_2-receptors) (4, 5). Unlike methysergide, ketanserin is devoid of intrinsic (agonistic) activity (6). These have prompted us to investigate the interaction of 5HT and ketanserin in vas deferens. We now describe the effects of ketanserin on 5HT-induced contractions and, furthermore, on the modulation by 5HT of twitch contractions in isolated rat vas deferens.

Materials and Methods

Wistar rats weighing 150–200 g were sacrificed by blows on the head and exanguination. The vasa deferentia were removed and cleaned of adhering tissues. The entire vas deferens was suspended in a 30 ml organ bath containing Krebs-Henseleit solution of the following composition: NaCl, 117.6; KCl, 5.4; MgSO_4, 0.56; CaCl_2, 2.5; NaH_2PO_4, 1.2; NaHCO_3, 24.99; and glucose, 11.1 mM. The bath fluid was maintained at 37°C and was bubbled with a mixture of 95% O_2–5% CO_2. A resting tension of 0.5 g was applied to the preparation, and the contractile responses were isometrically measured by a force displacement transducer (SB-1T, Nihon Kohden).

In an attempt to clarify if 5HT affects the sensitivity of postjunctional α-adrenoceptors of vas deferens, the contractile effects of 1-phenylephrine were determined before and after the addition of 5HT to the bath. In the experiments in which effects on the contraction induced by 5HT were examined, 5HT was applied to the organ bath 5 min after
pretreatment of the tissue with each antagonist. Because of the appearance of tachyphylaxis as previously reported (7), the effects of antagonists were determined by using different preparations.

Twitch contractions were elicited by electrical field stimulation (1 msec, 0.1 Hz, supramaximal voltage) (8) and were isometrically measured as described above. After the effects of 5-HT on the twitch contractions were observed and the tissue was washed out, influences of each antagonist on the effects of 5-HT were examined. In the concentration-response studies, 5-HT was cumulatively added to the bath (9) before and 5 min after the pretreatment of the tissue with each concentration of antagonist. The successive 5-HT concentration-effects curves were obtained at intervals of approximately 40 min.

The following drugs were used: ketanserin (R41468, Janssen), methysergide maleate (Sandoz), prazosin hydrochloride (Pfizer), 5-hydroxytryptamine creatinine sulphate (serotonin, Tokyo Kasei), 1-phenylephrine hydrochloride (Sigma), phentolamine mesylate (Ciba), propranolol hydrochloride (Kyowa Hakko), morphine hydrochloride (Takeda), promethazine hydrochloride (Yoshitomi), cimetidine (Fujisawa), atropine sulphate (Wako) and indomethacin (Sigma).

Results

1. 5HT-induced contractions and the effects of antagonists: 5HT (10^{-5} M) produced a phasic contraction, followed by rhythmic contractions. The height of the phasic component was about one-third of the contraction produced by a submaximal concentration of 1-phenylephrine (10^{-5} M). 5HT (10^{-6} M) neither potentiated nor inhibited the contraction induced by 1-phenylephrine (10^{-6}–10^{-5} M) (Fig. 1).

Figure 2 shows the effects of ketanserin, prazosin and methysergide on the 5HT-induced contractions. Ketanserin at 10^{-6} M inhibited both the phasic and the following rhythmic contractions, and 10^{-7} M of it almost completely abolished the 5HT-induced responses. Prazosin (10^{-6} M) partly inhibited the phasic contraction and, to a greater extent, reduced the amplitude of rhythmic contractions. Methysergide (10^{-6} M) partly inhibited the phasic and rhythmic contractions. Methysergide (10^{-6} M) partly inhibited the phasic and rhythmic contractions. Methysergide (10^{-6} M) partly inhibited the phasic and rhythmic contractions. Methysergide (10^{-6} M) partly inhibited the phasic and rhythmic contractions. Methysergide (10^{-6} M) partly inhibited the phasic and rhythmic contractions.
contractions, but the latter contractions were relatively resistant to methysergide.

2. Effects of 5HT on twitch contractions in the absence and in the presence of antagonists: Electrical field stimulation of rat vas deferens by squarewave pulses (1 msec, 0.1 Hz, supramaximal voltage) produced regular contractions (twitch contractions) of the tissue. 5HT, at concentrations of $10^{-7}$ to $10^{-5}$ M, elicited variable effects on twitch contractions, in which slight augmentation or attenuation of the amplitude were observed in different preparations. At the same time, 5HT produced a small and transient (30 to 90 sec) increase in the basal tension, corresponding to the phasic contraction as observed in the preceding paragraph. However, the changes in the amplitude of twitch contractions induced by 5HT were substantially minimal.

Ketanserin ($10^{-7}$ to $10^{-6}$ M) and prazosin ($10^{-7}$ to $10^{-6}$ M) per se slightly decreased the amplitude of twitch contractions. In the vas deferens treated with ketanserin ($10^{-7}$ to $10^{-6}$ M), 5HT ($10^{-5}$ M) promptly attenuated the amplitude of twitch contractions (Fig. 3). In the presence of prazosin ($10^{-6}$ M) or methysergide ($10^{-5}$ M), on the other hand, such attenuation by 5HT of the amplitude of twitch contractions was not observed (Fig. 4). Figure 5 shows the effects of ketanserin, methysergide and prazosin on the changes in twitch contractions produced by varying concentrations of 5HT. In the vas deferens pretreated with ketanserin ($10^{-8}$ to $10^{-6}$ M), 5HT ($10^{-7}$ to $10^{-5}$ M) consistently attenuated the amplitude of twitch contractions, and especially in the presence of $10^{-7}$ to $10^{-6}$ M of ketanserin, the attenuation by 5HT exhibited concentration-dependency. The effects of methysergide were inconsistent and variable; i.e. $10^{-7}$ M of methysergide tended to reduce the twitch contractions, but $10^{-5}$ M of it tended to augment them. Prazosin at concentrations of $10^{-8}$ to $10^{-7}$ M did not affect the concentration-effects curves for 5HT, whereas $10^{-6}$ M of it showed a tendency to augment the twitch contractions.

Experiments were carried out to determine whether 5HT-induced inhibition of twitch contractions observed in the presence of ketanserin was mediated by already clarified receptors or prostaglandin release. Neither antagonists examined nor indomethacin, an inhibitor of prostaglandin biosynthesis, affected the suppressing action of 5HT on the twitch contractions in the presence of ketanserin ($10^{-7}$ M) (Table 1).

**Discussion**

Nishino et al. (10) reported that 5HT-induced contractions in rat vas deferens were blocked by phentolamine, reserpine and imipramine, and suggested that 5HT induces contractions via the release of noradrenaline. On the other hand, it was suggested (11) that in guinea-pig vas deferens, the initial phasic component of 5HT-induced con-
tractions is mediated by postjunctional 5HT-receptors, but that the sustained component involves a presynaptic site of action through noradrenaline releases. More recently, Hay and Wadsworth (7) postulated that in rat vas deferens, the phasic component of 5HT-induced contractions is mediated by postjunctional 5HT-receptors, while the following rhythmic component is mediated not only by postjunctional 5HT-receptors but also by noradrenaline released from neuronal stores. From these reports, it seems apparent that 5HT induces contractions of vas deferens via combined effects which involve postjunctional 5HT-receptors and noradrenaline released from nerve endings, although the precise mechanism involved is still controversial. In the present experiments, ketanserin almost completely abolished not only the initial phasic but also the following

![Fig. 5. Effects of ketanserin (left) methysergide (center) and prazosin (right) on the 5HT-induced changes in twitch contraction amplitude of the rat vas deferens. 5HT was cumulatively added to the bath before and 5 min after each concentration of antagonists was treated, and the concentration-effects curves for 5HT were determined. The amplitude before and after antagonists were expressed as 100%. Each point represents the mean±S.E. of 5 experiments. Statistically significant (*P<0.05, **P<0.01) difference (paired t-test).]

Table 1. Effects of several antagonists on the 5HT-induced inhibition of rat vas deferens twitch contraction in the presence of ketanserin (10^-7 M)

| Treatment(a) | % Inhibition(b) (N)(c) |
|--------------|------------------------|
| Control      | 41.4±2.0 (8)           |
| Phentolamine | 40.0±4.0 (4)           |
| Propranolol  | 40.6±4.0 (4)           |
| Methysergide | 43.2±2.7 (4)           |
| Morphine     | 43.7±2.6 (4)           |
| Promethazine | 44.2±1.1 (4)           |
| Cimetidine   | 39.9±5.8 (4)           |
| Atropine     | 39.4±6.2 (4)           |
| Indomethacin | 37.9±3.7 (4)           |

(a) Vasa deferentia were pretreated with drugs for 5 min before the addition of 5HT (10^-6 M). (b) Values are presented as mean±S.E. (c) No. of experiments are indicated in parentheses. Note that none of the drugs examined affected the control responses.
rhythmic contractions, whereas the inhibitions by prazosin and methysergide were incomplete. One possible explanation of the complete inhibition by ketanserin might be that ketanserin possesses not only 5HT antagonistic activity but also, at relatively higher concentrations, α-adrenoceptor blocking activity (6). In fact, our unpublished observations indicate that the α-blocking activity of ketanserin is about one-third as potent as that of prazosin in rat vas deferens. That is, pA₂-values for ketanserin and prazosin against 1-phenylephrine were 7.72±0.06 (n=6) and 8.22±0.10 (n=5), respectively. Our present results, furthermore, indicate that 5HT-receptors which are located on rat vas deferens and elicit contractions are the 5HT₂-type ones, since ketanserin has high binding affinity for 5HT₂-receptors without any affinity for 5HT₁-receptors (4).

Many studies have shown that noradrenaline is the main transmitter in vas deferens (12-14), although the involvement of other mediators has been suggested (15, 16). Gillespie and McGrath (2) reported that in the rat vas deferens subjected to train pulses of nerve stimulation, lysergic acid diethylamide (LSD) attenuated the twitch component of the contraction, while LSD augmented the second component of contraction, presumably via the mechanism of LSD binding to 5HT receptors. The modulation by 5HT of neurotransmission in vas deferens was also reported by Fuenmayer et al. (3) who reported that 5HT enhanced the contractile responses due to high frequency (5-100 Hz) transmural stimulation. In our present investigation in which the twitch contractions of rat vas deferens were produced by low frequency (0.1 Hz) field stimulation, 5HT (10⁻⁷-10⁻⁵ M) per se did not substantially affect the amplitude of twitch contractions. These differences of the effects of 5HT might be attributed to the experimental conditions such as the frequency of stimulation.

The present results demonstrated that 5HT, in the presence of ketanserin, inhibited the twitch contractions of rat vas deferens subjected to field stimulation at 0.1 Hz. On the other hand, such inhibition was not observed in the presence of prazosin, and the inhibition in the presence of methysergide was minimal and observed only at its lower concentration. From the facts that 5HT contracts rather than relaxes the vas deferens and that 5HT does not affect the contractile responses induced by an α-adrenoceptor agonist, it is suggested that the inhibition by 5HT of twitch contractions in the presence of ketanserin is the presynaptic inhibition by 5HT of neurotransmitter release from the nerve endings. It has already been reported (17, 18) that in canine vessels, 5HT-receptors exist, stimulation of which depresses sympathetic tone by inhibiting the release of transmitter during nerve depolarization. Our present observation in rat vas deferens might be similar to the results in canine vessels.

In order to define the nature of the receptor by which 5HT inhibits the twitch contractions in the presence of ketanserin, we have examined several classical antagonists on the 5HT-induced inhibition. However, the results could rule out the involvement of α- or β-adrenoceptors, classical D- or M-5HT-receptors, H₁- or H₂- histamine receptors and muscarinic receptors. Moreover, the mediation of prostaglandin release was unlikely as indomethacin was ineffective. Further studies are needed to elucidate the mechanism by which 5HT inhibits twitch contractions.

Why was the twitch contractions depressed by 5HT in the presence of ketanserin? It is clear that the effect of ketanserin cannot be attributed to its α-blocking action since prazosin was ineffective. We suspect that 5HT has dual (excitatory and inhibitory) effects on the nerve transmission of rat vas deferens, and ketanserin might depress only the excitatory effect of 5HT without affecting the inhibitory one. This hypothesis is in accordance with the previous findings (2, 3) that 5HT and LSD either potentiate or inhibit the nerve-simulated contractions. The inhibition by cocaine of 5HT-induced noradrenaline release from sympathetic nerve endings has been reported in some organs (18, 19). Such inhibition by cocaine is considered to be due to its inhibitory action on the uptake of 5HT into the sympathetic pathway.
nerve endings (18). This raises the possibility that ketanserin might inhibit the uptake of 5HT into the nerve endings, thereby attenuating the 5HT-induced noradrenaline release. It is, however, unlikely that the inhibition by ketanserin of the 5HT-induced excitatory effects on nerve transmission is directly mediated by cocaine-like uptake inhibition, since ketanserin does not elicit supersensitivity of contractility as has been reported for cocaine (20). The inhibition by ketanserin might be ascribed to the blockade of 5HT2-receptors which are located presynaptically and augment nerve transmission. If so, the inability of higher concentrations of methysergide could be explained by its agonistic action (6).

Most recently, Fozard (21) reported that the compound MDL 7222, which is devoid of affinity for the 5HT2-receptor, antagonized the neuronal excitatory effect of 5HT in rabbit heart. Therefore, there remains the possibility that in rat vas deferens, ketanserin inhibited the neuronal excitation at another site of action rather than at 5HT2-receptors.

In conclusion, our present studies demonstrated that 5HT, in the presence of ketanserin, attenuated the amplitude of twitch contractions of rat vas deferens subjected to low frequency field stimulation. It was suggested that 5HT has dual (excitatory and inhibitory) effects upon the nerve transmission of rat vas deferens, which could be differentiated by ketanserin.

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