Probable Pre- and Postsynaptic Modifications by 5-Hydroxytryptamine of Contractile Responses to Electrical Stimulation of Isolated Guinea-Pig Vas Deferens

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Abstract—Two kinds of electrical stimulation, low frequency stimulation (5 Hz, 1 msec, 5 pulses, every 20 sec) and high frequency stimulation (30 Hz, 0.05–0.1 msec, 10 pulses, every 20 sec), produced contractions in the isolated guinea-pig vas deferens. These responses were blocked by tetrodotoxin but not hexamethonium. Phentolamine potentiated the contractions produced by low frequency stimulation, while it reduced the contractions produced by high frequency stimulation. Diametrically, 5-hydroxytryptamine reduced the contractions produced by low frequency stimulation, while it potentiated the contractions produced by high frequency stimulation. These inhibitory and potentiating actions of 5-hydroxytryptamine were reversed by cyproheptadine and 2-bromolysergic acid diethylamide. Moreover, that 5-hydroxytryptamine produced a depolarization of the smooth muscle membrane was shown by the sucrose gap technique. The results suggest that a 5-hydroxytryptamine receptor exists pre- and postsynaptically in the neuroeffector transmission of the guinea-pig vas deferens, that the stimulation of the presynaptic receptor by 5-hydroxytryptamine inhibits the release of a transmitter from noradrenergic nerves, and that the stimulation of the postsynaptic receptor by a high concentration of 5-hydroxytryptamine produces a depolarization of the smooth muscle membrane, and this relates to the potentiation of contractile responses.

5-Hydroxytryptamine (5-HT) has been shown to produce at least two effects on smooth muscle preparations. First, it has been reported to cause contractions in isolated dog femoral artery and saphenous vein (1, 2) and isolated rabbit ear artery (3). Secondly, 5-HT can inhibit motor responses to field stimulation of nerves in the dog saphenous vein (4). In these studies, the first case is thought to be due to an action of 5-HT on tryptaminergic receptors on the smooth muscle, and the second case is thought to be due to an action of 5-HT on tryptaminergic receptors on the presynaptic nerve terminal.

As regards to the neuroeffector transmission on the vas deferens, it is evident from the literature (5, 6) that presynaptic effects of adrenergic antagonists are predominant at relatively low frequencies of nerve stimulation (1–5 Hz), and postsynaptic effects of these agents are predominant at relatively high frequencies of nerve stimulation (25 Hz or higher). We focused our attention on the difference of responses to the low and high frequency nerve stimulations and investigated the action of 5-HT on guinea-pig vas deferens by using these frequencies of nerve stimulation. It was hoped that the pre- and post-synaptic actions of 5-HT on the neuroeffector transmission in guinea-pig vas deferens could be distinguished by two kinds of electrical stimulation parameters.

Materials and Methods

Male guinea-pigs weighing from 330 to 360 g were stunned by a blow on the head...
and bleb. The whole vas deferens was dissected and mounted in an organ bath. The prostatic end of the organ was tied to the bottom of the organ bath, and the epididymal end was attached to a transducer with silk thread. The organ bath contained 10 ml of Tyrode solution bubbled with a mixture of 95% oxygen and 5% carbon dioxide and was maintained at 32°C. The composition of Tyrode solution (mM) was as follows: NaCl, 137.0; KCl, 2.7; CaCl₂, 1.4; MgCl₂, 1.1; NaH₂PO₄, 0.4; NaHCO₃, 12.0; glucose, 5.6. Contractile responses were measured using an isotonic transducer (TD-112S, Nihon Kohden) at a load of 500 mg and were displayed on a chart recorder.

The preparations were stimulated with square wave pulses from an electronic stimulator (SEN-1101) with an isolator unit (SS-101J, Nihon Kohden). In these experiments, two arrangements for electrical stimulation were used. Low frequency stimulation was carried out with two platinum ring electrodes which were placed around the prostatic end of the muscle where the motor nerves enter the tissue. The stimulation consisting of 5 Hz, 1.0 msec, 5 pulses, and supramaximal voltage was applied at a 20 sec interval. Contractions elicited by this stimulation were magnified five-fold by an amplifier. High frequency stimulation was carried out with two platinum straight electrodes (35 mm in length), one of which passed through the lumen of the vas deferens, and the other was placed outside in parallel. The stimulation consisting of 30 Hz, 0.05–0.1 msec, 10 pulses, and supramaximal voltage was applied at a 20 sec interval. Contractions elicited by this stimulation were magnified two-fold by an amplifier.

In order to obtain information about the electrical effect of 5-HT on the vas deferens, the sucrose gap technique was used. The apparatus shown in Fig. 1, consists of three compartments: A, B and C. Adhering to each side of compartment B was a rubber membrane. Vas deferens of about 40 mm in length was inserted into a small hole, which was bored at the center lower part of compartment B, through the rubber membrane and exposed to 1/7 M KCl solution, 10% sucrose and Tyrode (test) solution. The epididymal half and the prostatic half of the vas deferens were exposed to KCl solution and test solution, respectively. In this method only, the sucrose solution was circulated. The flow rate of the sucrose solution was approximately 0.2 ml/min and kept constant throughout an experiment. The Ag/AgCl electrodes which were insulated except for the tip were located in the test solution and KCl solution, respectively. Tyrode (test) solution and KCl solution were bubbled with a mixture of 95% oxygen and 5% carbon dioxide in compartments A and C. The whole apparatus was maintained at 32°C. Mechanical recording was obtained from the prostatic end, which was connected with thread to the isotonic transducer. Electrical recording was obtained by using a micro-amplifier (MEZ-8201) and DC-amplifier (AD-640G, Nihon Kohden).

The following drugs were used and their concentrations were expressed as micromolar in the experiments: 5-hydroxytryptamine creatinine sulfate (Wako Pure Chemicals), hexamethonium bromide (Merck), tetrodotoxin (Seikagaku Kogyo), phentolamine mesylate (Takeda Yakuhin), cyproheptadine hydrochloride (Sigma), methysergide hydrogen maleinate (Sandoz) and D-2-bromolysergic acid diethylamide (Sandoz).
Results

Low and high frequency stimulations:
Low and high frequency stimulations of the guinea-pig vas deferens produced contractile responses similar to the type defined by Swedin (7) as "rapid twitch" responses (Fig. 2A and 2B). The responses continued for 5 to 6 hr, but declined steadily with time. Hexamethonium (50 μM) did not reduce the responses to stimulations at both frequency, showing that the nerves involved were postganglionic. Tetrodotoxin (1.5 μM) abolished these responses completely, confirming that the abolition was likely to be an indirect action on the smooth muscle (Fig. 2A and 2B). The contractile responses recovered by washing the preparations for about 1 hr. The α-adrenergic blocking agent, phentolamine (60 μM) potentiated the size of the contractile responses to low frequency stimulation to 225.9±16.6% of the control value (n=10). The potentiating effect of phentolamine remained even after the tissue was washed with phentolamine-free Tyrode solution for 30 min (Fig. 2C). On the contrary, phentolamine reduced the size of contractile responses to high frequency stimulation by 88.2±3.5% (n=10). The inhibitory action of phentolamine was reversed by washing the preparations for a few minutes (Fig. 2D).

The difference in the effect of phentolamine on these contractile responses was not due to the difference in the stimulation arrangement, but due to the difference in frequency of stimulation. For instance, if the stimulation consisting of 5 Hz, 1.0 msec, 5 pulses and supramaximal voltage was applied to preparations in the arrangement made of two

![Fig. 2](image-url)

Fig. 2. The effects of hexamethonium (C₆), tetrodotoxin (TTX) and phentolamine on contractile responses of isolated guinea-pig vas deferens to low frequency stimulation (A and C) and high frequency stimulation (B and D). • above each drug indicates the addition of the drug to the bath. • above W in each trace indicates washing from the bath.
platinum straight electrodes, the potentiation of contractions by phentolamine was also observed. Similarly, if the stimulation consisting of 30 Hz, 0.05–0.1 msec, 10 pulses and supramaximal voltage was applied to preparations in the arrangement made of two platinum ring electrodes, the inhibition of contractions by phentolamine was also observed. However, the effect of phentolamine under these conditions of stimulation was less marked as compared with that of phentolamine under the conditions of stimulations as described in the Methods section.

The effect of 5-hydroxytryptamine on contractile responses to low and high frequency stimulations: The contractions of the vas deferens caused by low frequency stimulation were reduced by 5-HT (Fig. 3). At the concentration of 0.3 \( \mu \text{M} \), 5-HT reduced the size of the contractile responses by 32.5±6.3% (\( n=10 \)), while at the concentration of 3 \( \mu \text{M} \) reduced, it reduced the size by 15.5±5.4% (\( n=10 \)) (Fig. 5). The reduction of contractile responses by 5-HT was not in a concentration-dependent manner. In almost all preparations, the contractile responses were completely abolished by 5-HT (30 \( \mu \text{M} \)), and the inhibitory action lasted for as long as 5-HT remained in the bath. The contractile response recovered rapidly after the tissue was washed with 5-HT-free Tyrode solution. The threshold concentration for this action of 5-HT was approximately 0.0003 \( \mu \text{M} \) (Fig. 5).

The contractile response to high frequency stimulation was potentiated by 5-HT in a concentration-dependent manner (Figs. 4 and 5). The threshold concentration for this potentiating action was 0.3 \( \mu \text{M} \), which was four orders greater than the threshold con-

![Fig. 3. The inhibitory effect of 5-HT on contractile responses to low frequency stimulation. • above 5-HT indicates addition of 5-HT to the bath. • above W in each trace indicates washing from the bath.](image)

![Fig. 4. The potentiating effect of 5-HT on contractile responses to high frequency stimulation. • above 5-HT indicates addition of 5-HT to the bath. • above W in each trace indicates washing from the bath.](image)
concentration for the inhibitory action of 5-HT on contractile responses to low frequency stimulation. The potentiating action of 5-HT was rapid in onset and lasted for as long as 5-HT remained in the bath, but on washing it readily disappeared. Such an action pattern was marked especially at high concentrations of 5-HT. At the concentration of 30 μM, 5-HT potentiated the size of contractions to 168.9±8.1% of the control level (n=10).

Although the inhibitory action of 5-HT on contractile responses to low frequency stimulation was obtained in almost all preparations, the potentiating action of 5-HT on contractile responses to high frequency stimulation was not always observed in all preparations. In the present study, 43 guinea-pigs were used for the experiments on contractile responses to high frequency stimulation. In 8 out of the 43 guinea-pig preparations, no potentiating action of 5-HT on contractile responses to high frequency stimulation was observed. The results from such preparations were ruled out in Figs. 4 and 5.

Effects of antagonists on the inhibitory and potentiating actions of 5-hydroxytryptamine on contractile responses to low and high frequency stimulations: At the concentration of 10 μM, cyproheptadine and 2-bromolysergic acid diethylamide (BOL) themselves potentiated the size of contractions to low frequency stimulation; but at this concentration, methysergide itself had no effect on them.

The inhibitory action of 5-HT (30 μM) on contractile responses to low frequency stimulation was reversed by cyproheptadine (10 μM) and BOL (10 μM). The reverse effect of methysergide (10 μM) was very weak (Fig. 6). Even at the concentration of 30 μM, it did not restore the contraction to the control level. Therefore, it was evident that the reverse effect of methysergide on the inhibitory action of 5-HT was very weak as compared with those of cyproheptadine and BOL.

Effects of antagonists on the potentiating action of 5-HT on contractile responses to high frequency stimulation were examined at the same concentration as that used in experiments of low frequency stimulation. At the
concentration of 10 μM, methysergide and BOL themselves had little or no effect on the size of contractile responses to high frequency stimulation; but at this concentration, cyproheptadine itself produced a 10-20% potentiation of the responses. As shown in Fig. 4, 5-HT (30 μM) potentiated the contractions by high frequency stimulation; and against this potentiation, methysergide and cyproheptadine had no effect at the concentration used (10 μM), while BOL reversed the potentiating action of 5-HT (Fig. 7). However, at the concentration of 30 μM, cyproheptadine showed evidently the reverse effect on the potentiating action of 5-HT.

The effect of 5-HT in concentrations from 3.0–30.0 μM produced a depolarization of the smooth muscle in a concentration-dependent manner, although there was a considerable variation in sensitivity among the preparations. Within the range of these concentrations, 5-HT did not produce any contraction.

**Discussion**

In order to distinguish pre- and postsynaptic actions of 5-HT on neuroeffector transmission in the guinea-pig vas deferens, two kinds of electrical stimulation, low and high frequency stimulations, were used in this study. From the results of experiments with tetrodotoxin and hexamethonium, it is evident that the contractions produced by both frequency stimulations were mediated by activation of postganglionic nerves.

Phentolamine potentiated contractile responses to low frequency stimulation and reduced contractile responses to high frequency stimulation. According to Langer (8), the potency of phentolamine as an antagonist on the postsynaptic (α1) receptor which mediates the responses to the effector organ and the presynaptic (α2) receptor which mediates the inhibition of noradrenaline release during stimulation, is approximately equal. Therefore, it is considered...
that the potentiation of contractile responses to low frequency stimulation is a presynaptic effect of phentolamine, and the inhibition of contractile responses to high frequency stimulation is postsynaptic effect of this compound. This is in agreement with the observation of Drew (6) that the twitch response of the rat isolated vas deferens to low frequency motor nerve stimulation is potentiated by phentolamine and also in agreement with the observation of Birmingham and Wilson (5) that phentolamine abolished contractile responses elicited by transmural stimulation of 25 Hz frequency and at the same time blocked the response to added noradrenaline, indicating that this is a postsynaptic effect of phentolamine. These effects of phentolamine on contractile responses suggest that responses to low frequency stimulation are sensitive to the presynaptic effect of phentolamine, while the response to high frequency stimulation is sensitive to the postsynaptic effect of phentolamine.

In the present study, 5-HT inhibited the contractile responses to low frequency stimulation and potentiated the contractile responses to high frequency stimulation. The action of 5-HT is diametrically related to that of phentolamine. McGrath (9) reported that in the tissues where lysergic acid diethylamide (LSD) itself exerts a motor action, the net effect seen may be a balance between pre-junctional inhibition and postjunctional facilitation, and this particularly applied to vas deferens. Since 5-HT has the same action as LSD in the smooth muscle of isolated tissues, it will be possible to distinguish pre- and postsynaptic effects of 5-HT by suitably changing the parameters of electrical stimulation as described in the Methods. The results in the present experiments suggest that the inhibitory action of 5-HT on contractile responses to low frequency stimulation is due to the presynaptic effect of 5-HT, and the potentiating action of 5-HT on contractile responses to high frequency stimulation is due to the postsynaptic effect of 5-HT. The former is supported by the fact that in the isolated dog saphenous vein, a 5-HT receptor exists presynaptically, and stimulation of this receptor by a low concentration of 5-HT inhibited the release of noradrenaline from noradrenergic nerves (4); and the latter is thought to relate to a depolarization of the smooth muscle membrane. In the experiments of high frequency stimulation, the threshold concentration for the potentiating action of 5-HT was 3.0 μM (Fig. 5). Meanwhile, also in the experiments in which the electrical effect of 5-HT was tested, the threshold concentration of 5-HT for depolarizing smooth muscle membrane was 3.0 μM (Fig. 8). The accordance of these concentrations suggests that the potentiating action of 5-HT on contractile responses to high frequency stimulation is due to depolarization of smooth muscle membrane. Sjöstrand (10) investigated the effect of 5-HT on the electrical and mechanical responses of the guinea-pig vas deferens to nerve stimulation and described the results as follows: 5-HT produced a depolarization of smooth muscle membranes; because of this, the membrane came closer to the threshold for firing of action potentials; the action potentials also increased in size in the presence of the 5-HT, and the contractions became larger. The concentration range of 5-HT at which Sjöstrand observed the depolarization was approximately the same as that in our experiments. Therefore, it should be possible to apply these explanations to explain the results in our experiments.

It is natural to consider that the effect of 5-HT on smooth muscle membrane exerts some sort of change in the contractile responses to low frequency stimulation. As shown in Fig. 5, the curve representing the relationship between the inhibitory action of 5-HT and the concentration of 5-HT was not concentration-dependent. There was a shoulder at the concentration of 3.0 μM. Considering that this point is the threshold concentration for the potentiating action of 5-HT on contractile responses to high frequency stimulation, it is possible to say that the shoulder indicates the effect of 5-HT on the smooth muscle membrane. At still higher concentration (10–30 μM), this effect of 5-HT seemed to be denied by the presynaptic effect of 5-HT.

The results obtained in the experiments on antagonists showed that methysergide did
not reverse as markedly as cyproheptadine and BOL, the inhibitory effect of 5-HT on transmitter release in the electrically stimulated vas deferens. A similar result was reported by McGrath (11) who found that methysergide has no effect on the inhibitory effect of 5-HT on noradrenaline release in the stimulated saphenous vein strip. Moreover, Martinez and Lokhandwala (12) reported that 5-HT inhibits sympathetic neurotransmission to the myocardium via an action on tryptaminergic receptors which may be located on sympathetic nerve terminal and that these receptors can be selectively blocked by cyproheptadine but not by methysergide. These facts suggest that methysergide can not reverse the action of 5-HT or its reverse action is very weak at the tryptaminergic receptors on the postganglionic sympathetic nerve terminal.

In experiments of high frequency stimulation, methysergide and cyproheptadine showed no effect on the potentiating action of 5-HT, and this suggests that in the case of methysergide, it does not reverse the action of 5-HT in the smooth muscle membrane of the guinea-pig vas deferens; but in the case of cyproheptadine, another possibility can be considered. Although cyproheptadine itself produced the potentiating of contractile responses to high frequency stimulation, the pretreatment of preparations with cyproheptadine prevented the 5-HT-induced potentiation. Therefore, the result shown in Fig. 7 may show that the inhibitory action of cyproheptadine was denied by the potentiating action of this agent. In any case, it is conceivable that the ability of cyproheptadine to reverse the potentiating action of 5-HT is not as marked as that of BOL. Assuming this to be true, the order of potency of the three 5-HT antagonists in reversing the action of 5-HT was the same on contractile responses to low and high frequency stimulations. These facts suggest that at least two kinds of 5-HT receptors similar in nature to 5-HT antagonists exist in the guinea-pig vas deferens, and they exert functionally quite different actions on the effector organ.

The indirect action of 5-HT on smooth muscle preparations can be classified into two categories: a stimulant effect and an inhibitory effect. The stimulant effect is observed in contractions by a high concentration of 5-HT in isolated dog saphenous vein (1) and in the isolated muscularis mucosae of the guinea-pig oesophagus (13). The inhibitory effect is observed in contractions by electrical stimulation of isolated dog saphenous vein (4). In this study, the inhibitory action of 5-HT on contractile responses to low frequency stimulation is due to the inhibitory one. This is also supported by the fact that the inhibitory effect of 5-HT against nerve-induced contractions is more effective against low frequency and short trains of stimuli (4, 14). However, the potentiating action of 5-HT on contractile responses to high frequency stimulation does not appear to be the stimulant effect of an indirect action of 5-HT. Although responses mediated via the activation of 5-HT receptors in the peripheral nervous system show tachyphylaxis, the direct action of 5-HT on smooth muscle generally does not show it (15, 16). Also, in the present experiments, tachyphylaxis on the potentiating action of 5-HT to high frequency stimulation-induced contraction was not observed. Therefore, it is reasonable to infer that the potentiating action of 5-HT is not an indirect action on smooth muscle preparations.

Recently, it has been suggested that ATP acts as a co-transmitter with noradrenaline in the motor innervation of guinea-pig vas deferens. The contractile response to field stimulation of the vas deferens has been so far divided into first and second phases. Meldrum and Burnstock (17) have shown that the first phase of the neurogenic contractile response in guinea-pig vas deferens is purinergic. It is considered that the contractile response, especially to low frequency stimulation in the present study, corresponds to that of the first phase observed in their study. Therefore, it is supposed that the contractile response to low frequency stimulation is mediated by ATP.

In summary, the inhibitory action of 5-HT on contractile responses to low frequency stimulation demonstrated in this study is consistent with the concept of a reduction in the transmitter release, and this action of it is considered to be mediated via the
activation of tryptaminergic receptors which may be located on sympathetic nerve terminals. Another action of 5-HT is the potentiation of contractile responses to high frequency stimulation. 5-HT also produces a depolarization of the smooth muscle membrane. This depolarization is assumed to be due to the action of tryptaminergic receptors which may be located on the smooth muscle membrane. The potentiating action of 5-HT is considered to be related to the depolarization of the smooth muscle membrane.

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