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Biochemical Pharmacology, Vol. 28, pp. 951–952.

0006-2952/79/0315-0951 $02.00/0

A pre-synaptic inhibitory effect of 5-hydroxytryptamine on the electrically induced twitch response of the rat vas deferens

(*Received* 23 *June* 1978; *accepted* 13 *September* 1978)

There is now substantial evidence for the existence of presynaptic α-adrenoceptors located at noradrenergic varicosities both in the peripheral and central nervous system [1, 2]. Stimulation of these pre-synaptic α-adrenoceptors by α-adrenoceptor agonists results in a decreased release of noradrenaline whilst their blockade facilitates noradrenaline release by nerve impulses [3]. In addition to pre-synaptic α-adrenoceptors Stjärne and Brundin [4] have reported the existence of pre-synaptic β-adrenoceptors on noradrenergic nerve endings. Other pre-synaptic sites have been reported to be involved in a modulatory role on the noradrenergic nerve terminal and these include the receptors for dopamine [5], opiates [6], prostaglandins [7] and histamine [8].

It has recently been reported [9] that 5-hydroxytryptamine (5-HT) can induce an increase in the outflow of noradrenaline from sympathetic nerve terminals and that this effect may be mediated via pre-synaptic tryptamine receptors. The present study was therefore undertaken to investigate the effects of 5-HT on the electrically induced contractions or 'twitches' [10] of the rat isolated vas deferens, a preparation which has been used extensively to characterize pre-synaptic α-adrenoceptors [11–14] and presynaptic histamine receptors [8], to establish whether or not 5-HT has a modulatory role on transmitter release via a presynaptic site.

Vasa deferentia from male Wistar rats (200–250 g) were set up in organ baths and bathed in a Mg-free Krebs solution of the following composition (mM): NaCl 119, $CaCl_2$ 2.6, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2 and glucose 11.1, which was maintained at 37° and aerated with a mixture of 5% CO_2 in O_2. Silver electrodes were placed near the top and bottom of the tissue and the intramural nerves of the vas deferens stimulated according to Birmingham and Wilson [15] by square wave pulses of 3 msec duration, 20–25 V at a frequency of 0.1 Hz, provided by a Grass S4 stimulator. Isometric contractions were recorded using Devices transducers and two channel recorders.

5-HT produced a concentration dependent inhibition of the twitch response with an ID_{50} of 0.6 μM; this action was rapid in onset and readily reversed by washing with Krebs solution. A cumulative dose–response curve is shown in Fig. 1. The effect of the 5-HT antagonists cyproheptadine and methysergide on the inhibitory effect of 5-HT on the twitch response was investigated. Methysergide proved to be an effective competitive antagonist, producing a dose-dependent parallel shift of the 5-HT dose–response curve to the right (Fig. 1). Cyproheptadine, though producing antagonism of the 5-HT inhibitory response was not as effective as methysergide and at concentrations above 10 μM itself produced inhibition of the twitch response of the electrically stimulated vas deferens.

An inverse relationship between the inhibitory effect of 5-HT (2 μM), added 30 sec before stimulation, and stimulation frequency was observed; the lower the frequency the greater was the inhibitory effect of 5-HT (Fig. 2); this observation is indicative of a pre-synaptic site of action and correlates well with similar observations made for other agonists such as clonidine [11], noradrenaline [16], histamine [8] and ergometrine [17] acting on pre-synaptic receptor sites.

It has been reported [18–20] that a single pulse stimulation

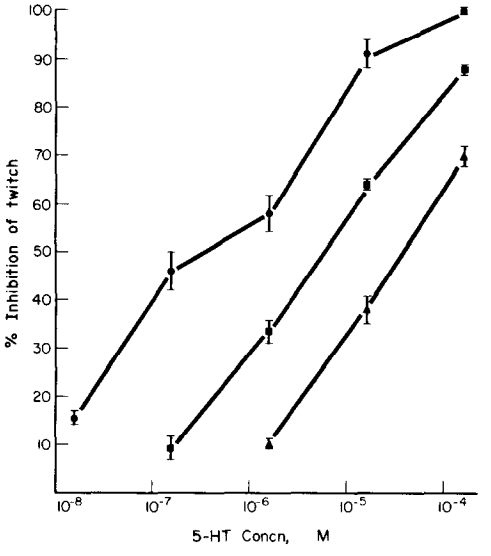

Fig. 1. The inhibition by 5-hydroxytryptamine (5-HT) of the electrically induced twitch response of the rat vas deferens and its antagonism by methysergide. ●——● Control 5-HT inhibitory response (9). ■——■ 5-HT response following a 10 min exposure to 20 μM methysergide (4). ▲——▲ 5-HT response following a 10 min exposure to 0.2 mM methysergide (4). Each point is the mean ± standard error of the mean. Figures in parentheses indicate the numbers of experiments carried out for each curve.

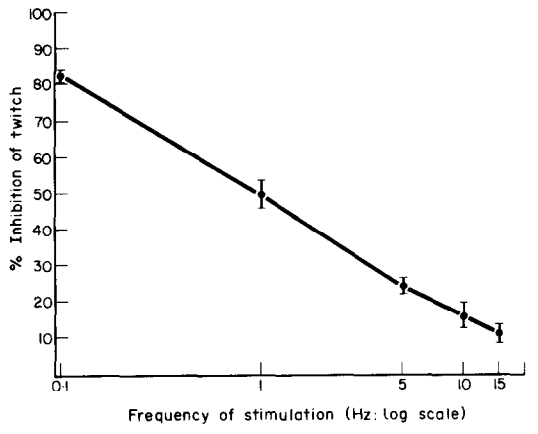

Fig. 2. The inhibitory effect of a standard (2 μM) concentration of 5-HT on the twitch response of the isolated rat vas deferens, showing the reduction in inhibition with increased frequency of stimulation. Each point is the mean ± S.E.M. from three experiments.

producing the twitch response of the vas deferens is largely nonadrenergically mediated and that endogenously released noradrenaline serves an inhibitory role upon motor transmission [21]. It is therefore possible that 5-HT may inhibit the twitch response of the vas deferens by evoking a release of noradrenaline which then produces a presynaptic inhibitory effect. Fozard and Mobarok Ali [9] have postulated the existence of cardiac pre-synaptic tryptamine receptors activation of which, by high concentrations of 5-HT, stimulates noradrenaline release whilst low concentrations of 5-HT produce a desensitization of the receptors [22]. The results obtained in the present study are in partial agreement with these observations in that 5-HT at low concentrations produced a dose-dependent inhibition of the twitch response of the isolated rat vas deferens stimulated at 0.1 Hz. However no tyramine-like excitatory effects due to the release of endogenous noradrenaline were seen even at high concentration of 5-HT. Support for the fact that 5-HT is acting pre-synaptically is provided by the results that the higher the frequency of stimulation the lower was the inhibition of the twitch response by 5-HT, a result which is in agreement with the observation that agonists at pre-synaptic receptors are more effective at low rather than at high frequencies of stimulation [23].

It is possible that 5-HT, like clonidine, inhibits the twitch response by activating pre-synaptic α-adrenoreceptors [16]. Alternatively, a specific 5-HT sensitive receptor may be located pre-synaptically, activation of which leads to a decrease in the outflow of transmitter. The second possibility would appear to be the more likely since methysergide exhibited a classical competitive antagonism on the 5-HT response. However, the question is clearly unresolved and work is at present underway to ascertain through which receptor system 5-HT produces its pre-synaptic inhibitory response, and to correlate these effects on the twitch response with the effects of 5-HT on overflow of [^{3}H]noradrenaline.

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