Br. J. Pharmac. (1973), **48**, 233–244.

Interaction between picrotoxin and 5-hydroxytryptamine in the superior cervical ganglion of the cat

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Summary

1. Electrophysiological techniques were utilized to study the actions of 5-hydroxytryptamine (5-HT) and picrotoxin on the superior cervical ganglion of the cat.

2. The intra-arterial administration of 5-HT to the ganglion elicited both depressant and excitatory actions. In low doses (0·01–0·5 μg) the amine produced a depression of ganglionic transmission. In larger doses (2–50 μg) it produced an excitation of ganglion cells (early discharge) and an initial enhancement of transmission, which was followed by depression. Picrotoxin (25–500 μg, i.a.) blocked the initial excitatory effects of 5-HT but did not block the depression. Picrotoxin did not antagonize the excitatory actions of injected cholinomimetic agents or potassium chloride.

3. In ganglia conditioned by repetitive stimulation of the preganglionic nerve (30 Hz for 30 s) 5-HT also elicited a late-occurring and very prolonged discharge on certain postganglionic nerves ('spinal') but not on others (external carotid). The late discharge was only partially depressed by picrotoxin.

4. Recordings from the surface of the superior cervical ganglion revealed that 5-HT produced three types of ganglionic potentials: (1) an initial transient depolarization which coincided with the early discharge, (2) a late-occurring, prolonged depolarization which coincided with the late discharge, and (3) a hyperpolarization which in some experiments accompanied the depression of transmission. The late depolarization and hyperpolarization were not observed in every experiment. Picrotoxin (25–500 μg) blocked the initial depolarization, but did not block the late depolarization or the hyperpolarization.

5. It is concluded the 5-HT can produce three distinct responses in the superior cervical ganglion: a depressant effect and two types of excitation. It seems likely that depression and excitation occur via the activation of different receptors, since picrotoxin selectively blocks the latter. The finding that picrotoxin is a 5-HT antagonist in peripheral ganglia raises the possibility that picrotoxin might also influence tryptaminergic mechanisms in the central nervous system.

Introduction

The excitatory actions of 5-hydroxytryptamine (5-HT) on autonomic ganglia are well known (see review by Trendelenburg, 1967). In 1954, Robertson showed that 5-HT could elicit a contraction of the cat's nictitating membrane by stimulating neurones in the superior cervical ganglion. Subsequently, it was demonstrated

that 5-HT depolarized ganglia (Reinert, 1960 ; de Groat & Volle, 1966a), produced firing in postganglionic nerves (Bindler & Gyermek, 1961 ; Gyermek & Bindler, 1962a, b ; de Groat, 1970) and facilitated ganglionic transmission (Trendelenburg, 1957 ; Hertzler, 1961). The ganglionic excitatory actions of 5-HT are resistant to competitive ganglionic blocking agents, such as hexamethonium and tetraethylammonium, but are reduced by morphine, cocaine (Gaddum & Picarelli, 1957 ; Gyermek & Bindler, 1962a), certain 5-HT analogues (e.g., 5-hydroxy-3-indoleacetamidine, Gyermek & Bindler, 1962a) and gamma aminobutyric acid (de Groat, 1970).

During an earlier investigation of the ganglionic interactions between gamma aminobutyric acid (GABA) and 5-HT (de Groat, 1970) it was observed that picrotoxin, a GABA-antagonist, blocked the excitatory effects of 5-HT. The present experiments were undertaken to examine this unexpected action of picrotoxin. In the course of these experiments further information was obtained about the actions of 5-HT at ganglionic synapses. It was discovered that 5-HT produced two types of ganglionic excitatory responses and elicited a depression as well as facilitation of ganglionic transmission.

Methods

Experiments were performed on cats anaesthetized with a mixture of sodium diallylbarbiturate (70 mg/kg), urethane (280 mg/kg) and monoethylurea (280 mg/kg).

After the patency of the airway was ensured by intubation of the trachea, a deep cervical well was prepared by removing the upper portions of the trachea, the oesophagus, and the larynx. The left superior cervical ganglion was exposed and the postganglionic nerve to the carotid bifurcation and external carotid artery was identified. The postganglionic nerve was dissected free from underlying tissues and cut at the level of the carotid bifurcation. In many experiments the postganglionic nerves to the cervical spinal roots were also isolated. The cervical sympathetic trunk was dissected free from the vagus nerve and common carotid artery and severed at a point approximately 2 cm above the clavicle. All major branches of the common carotid artery excepting those supplying the ganglion were tied. Skin flaps were secured to a metal frame and the exposed area was covered with warmed paraffin oil.

Drugs were administered through a 27-gauge needle, which was inserted into the common carotid artery and clamped to the supporting framework. The volume of the injection was 0·1–0·2 ml. All of the drugs were dissolved in a solution of 0·9% w/v NaCl (saline). Clotting in the needle was prevented by the prior administration of heparin (300 units intra-arterially).

The acutely-decentralized preganglionic nerves were mounted on bipolar silver or platinum electrodes and stimulated with rectangular pulses of 0·05 ms duration. Potentials evoked by drugs or by preganglionic stimulation were recorded from the surface of ganglia by means of bipolar silver–silver chloride electrodes. One electrode was placed in direct contact with the body of the ganglion ; the other on the crushed end of the postganglionic nerve (external carotid branch). Ganglion potentials were amplified by a resistance-coupled preamplifier and visualized on one beam of a dual beam oscilloscope. In all records an upward deflection of the

tracing indicates a negativity of the ganglion with respect to the crushed postganglionic nerve. Ganglionic negativity and positivity will be referred to as depolarization and hyperpolarization, respectively. Evoked postganglionic activity was recorded with bipolar platinum electrodes, amplified with a capacitance-coupled amplifier, and displayed on the second beam of the oscilloscope. Permanent records were made on moving photographic paper or film. The time base was provided by the speed at which the paper moved through the recording camera.

The following drugs were used: Acetylcholine chloride, atropine sulphate, bulbocapnine hydrochloride, cocaine hydrochloride, creatinine sulphate, 2-diethoxyphosphenylthioethyldimethylamine acid oxalate (217 AO), dihydroergotamine methanesulphonate (DHE), bicuculline hydrochloride, hexamethonium chloride (C_6), 5-hydroxytryptamine creatinine sulphate (5-HT), (±)-isoprenaline hydrochloride, methysergide bimaleate, (−)-noradrenaline bitartrate, picrotoxin, acetyl-β-methylcholine chloride (methacholine), oxotremorine hydrochloride. Doses are expressed as the salt and refer to i.a. administration unless otherwise indicated.

Results

Ganglionic potentials and postganglionic firing evoked by 5-hydroxytryptamine

The intra-arterial administration of 5-hydroxytryptamine creatinine sulphate (5-HT) to the superior cervical ganglion produced changes in the demarcation potential recorded between the surface of the ganglion and the crushed end of a postganglionic nerve (external carotid branch). Three types of waveforms were observed although not in every experiment: (1) an initial transient, negative potential (early depolarization), (2) a late-occurring, more prolonged negative potential (late-depolarization) and (3) a positive potential or ganglionic hyperpolarization. The different types of ganglionic potentials evoked by 5-HT are illustrated in Figure 1. As reported by previous investigators (Reinert, 1960; de Groat & Volle, 1966a) large doses of 5-HT (0·2–40 μg) invariably elicited an early depolarization which was accompanied by asynchronous firing on postganglionic nerves (Figure 2). The responses commenced 2–4 s after injection and persisted for 5–20 seconds. In 8 of 23 experiments the early depolarization was followed by the late depolarization, which was of low amplitude and not accompanied by postganglionic firing in the untreated ganglion (Figures 1C, 2). The duration of the

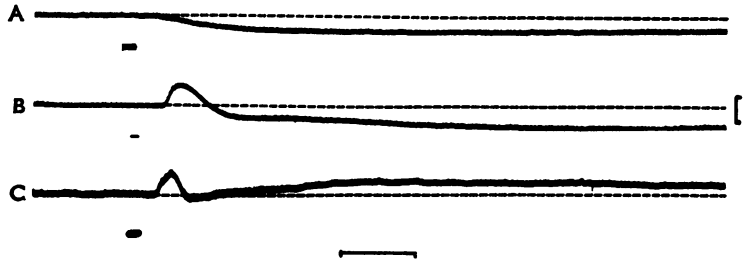

FIG. 1. Ganglionic potentials elicited by 5-hydroxytryptamine (5-HT). Records A and B, responses from the same experiment elicited, respectively, by 0·05 and 0·5 μg of 5-HT. Record C, response from a different experiment elicited by 10 μg of 5-HT. Injections are indicated by bar below each record. In this figure and all subsequent figures an upward deflection of the baseline indicates ganglionic negativity. Vertical calibration is 100 μV. Horizontal calibration is 10 seconds.

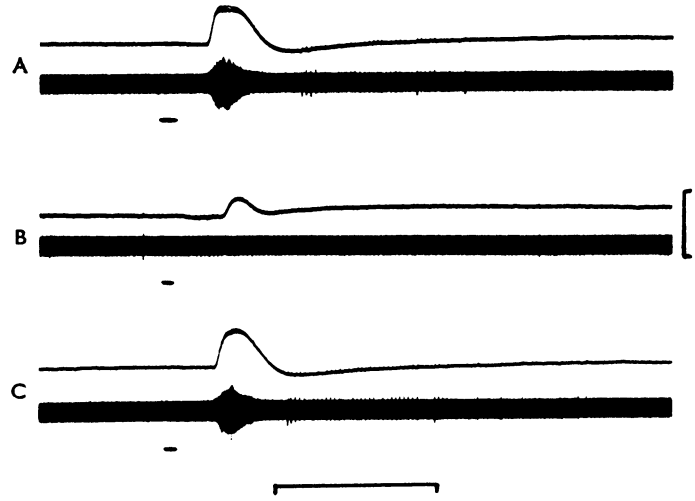

FIG. 2. Effect of picrotoxin (150 μg) on the ganglionic responses evoked by 5-hydroxytryptamine (5-HT, 5 μg). Top and bottom tracings of each record are, respectively, ganglionic potentials and postganglionic potentials. Recordings from external carotid postganglionic branch. Record A, response to 5-HT in the untreated ganglion. Record B, response to 5-HT 30 s after the administration of picrotoxin. Record C, response to 5-HT 15 min after picrotoxin. Vertical calibration represents 400 μV in upper tracing and 60 μV in lower tracing of each pair. Horizontal calibration is 10 seconds. Injection indicated by a bar below each record.

late-depolarization ranged between 1–3 minutes. In the remaining experiments the early depolarization was followed by a hyperpolarizing wave or by a return to the pre-injection base-line. The hyperpolarization could be of very short duration (5–10 s) as illustrated in Fig. 2A or of long duration (2–3 min) as shown in Figure 1B.

In 6 out of 23 experiments low doses of 5-HT (0·005–0·5 μg) elicited purely a hyperpolarizing potential (Figure 1A). The amplitude and duration of hyperpolarization were dose dependent, ranging to a maximum of 200 μV and 3 min, respectively. In the same experiments larger doses of 5-HT (0·5–20 μg) produced a biphasic response (depolarization–hyperpolarization) as described above. It should be noted however that biphasic responses to large doses of 5-HT were also observed in ganglia where low doses of 5-HT did not produce a detectable hyperpolarizing response.

Effect of repetitive preganglionic nerve stimulation on the postganglionic responses evoked by 5-hydroxytryptamine

High frequency stimulation (30–50 Hz) of the preganglionic input to the superior cervical ganglion of the cat produces a prolonged depolarization of the ganglion and enhances the postganglionic firing elicited by various ganglionic stimulating agents (Volle, 1962; Takeshige & Volle, 1964; Trendelenburg, 1967). In the present experiments repetitive preganglionic stimulation (30 Hz) enhanced the early discharge to 5-HT and unmasked a prolonged low amplitude discharge which persisted for 0·5–3 min (Figure 3). The late discharge was regularly observed (12 of 15 experiments) on the postganglionic nerves to the spinal roots ('spinal' nerves) but was rarely detected (3 of 18 experiments) on the postganglionic branches to the external carotid artery; although on both nerves the repetitive preganglionic stimulation enhanced the early discharge. The late discharge com-

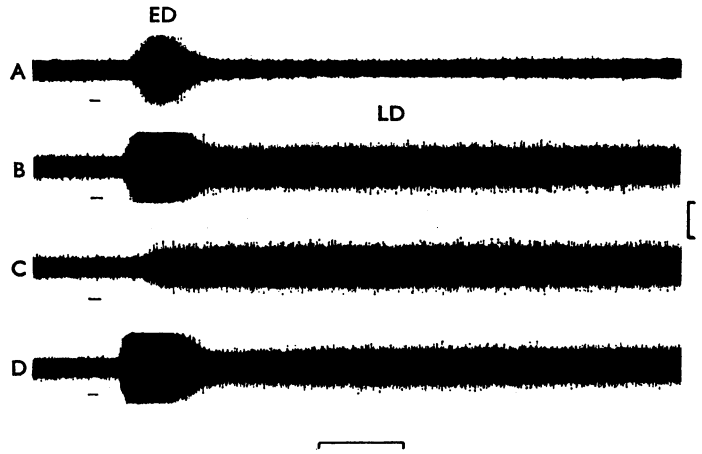

FIG. 3. Effect of picrotoxin and repetitive preganglionic nerve stimulation on the postganglionic discharge produced by 5-hydroxytryptamine (5-HT, 2·5 μg). Records A–D, recordings of asynchronous firing on a postganglionic nerve to the cervical spinal roots ('spinal branch'). Record A, early discharge (ED) to 5-HT in the untreated ganglion. Record B, response to 5-HT after repetitive stimulation of the preganglionic nerve at 30 Hz for 30 seconds. Response consists of an early discharge (ED) which is truncated and a late discharge (LD). Record C, block of the ED to 5-HT, 30 s after the administration of picrotoxin (75 μg). Record D, recovery of the ED 15 min after the administration of picrotoxin. Vertical calibration is 40 μV. Horizontal calibration is 10 seconds. Injection indicated by a bar below each record.

monly occurred at doses of 5-HT below the threshold for producing an early discharge and once unmasked, the late discharge could be elicited for the remainder of the experiment (3–5 hours). The administration of isoprenaline (2–10 μg), a catecholamine which depolarizes the superior cervical ganglion (de Groat & Volle, 1966a, b) also unmasked the late response to 5-HT on both external carotid (de Groat & Volle, 1966a) and 'spinal' postganglionic nerves. The effect of isoprenaline persisted for 10–20 min depending upon the dose administered.

Since 5-HT was administered in the form of a creatinine sulphate complex, we also tested for possible ganglionic excitatory actions of the creatinine sulphate moiety. Creatinine sulphate however, administered in doses as large as 50–100 μg, did not elicit a detectable early or late postganglionic discharge.

Effect of drugs on the ganglionic potentials and postganglionic firing evoked by 5-hydroxytryptamine

In experiments where antagonists were to be administered just prior (less than 1 min) to an injection of 5-HT, we routinely tested the effects of an injection of saline on the responses to 5-HT. It was noted in a few experiments (4 of 24) where the ganglionic circulation was sluggish, that an injection of 0·1–0·2 ml of saline 10–30 s before the administration of 5-HT reduced the ganglionic responses to the monoamine. The depression may be related to the observation that ganglia perfused with artificial media are much less sensitive to the excitatory actions of 5-HT than are ganglia with an intact circulation (Trendelenburg, 1957).

The administration of picrotoxin (25–500 μg) before the injection of 5-HT reversibly blocked the initial discharge (20 experiments ; Figs. 3 and 4) and reduced the amplitude of the initial ganglionic depolarization (8 experiments ; Figs. 2 and 5).

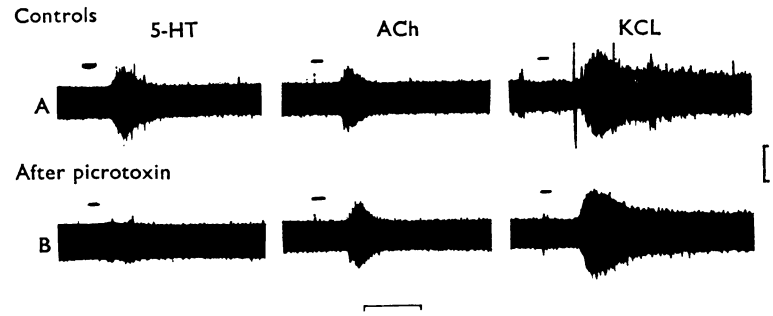

FIG. 4. Effect of picrotoxin on the postganglionic firing elicited by 5-hydroxytryptamine (5-HT, 40 μg), acetylcholine (ACh, 10 μg) and potassium chloride (KCl, 0·75 mg). Recordings on external carotid postganglionic branch. Top tracings (Record A, Controls), responses in the untreated ganglion. Bottom tracings (Record B, After picrotoxin), responses 1 min after the administration of picrotoxin ; 200 μg of picrotoxin administered prior to the injection of 5-HT and 500 μg of picrotoxin administered prior to the injection of ACh and KCL. All recordings are from the same experiment. Vertical calibration is 10 μV. Horizontal calibration is 5 seconds. Injections indicated by a bar above each record.

The blocking action of picrotoxin, which was apparent within 10–15 s after injection and persisted for 5–20 min, was surmountable on increasing the dose of 5-HT. The degree of blockade was related to the type of 5-HT responses being studied, e.g. the discharge elicited by 5-HT was blocked at lower doses of picrotoxin than was the ganglionic depolarization. Picrotoxin did not block the 5-HT-evoked hyperpolarization (2 experiments ; Figure 5).

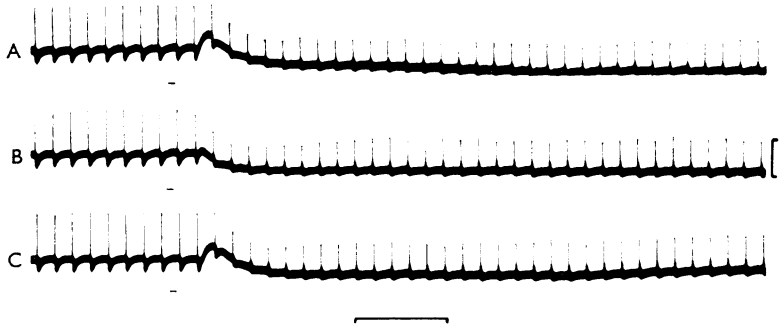

FIG. 5. Effect of picrotoxin (20 μg) on the ganglionic potentials and the depression of transmission produced by 5-hydroxytryptamine (5-HT) (0·5 μg). Ganglionic potentials evoked by submaximal preganglionic nerve stimulation at a frequency of 0·5 Hz. Record A, response to 5-HT in the untreated ganglion. Record B, response 30 s after the administration of picrotoxin. Record C, response 15 min after picrotoxin. Vertical calibration is 200 μV. Horizontal calibration is 10 seconds. Injections indicated by a bar below each record.

Doses of picrotoxin (25–100 μg) which were adequate to block the initial discharge to 5-HT did not depress the late discharge (Fig. 3) ; however, larger doses of picrotoxin (100–500 μg) reduced the late discharge but never completely blocked it. Picrotoxin (25–500 μg) did not depress the postganglionic responses elicited by acetylcholine (10–50 μg), potassium chloride (0·5–1·5 mg) (Fig. 4) or by muscarinic ganglionic stimulants, methacholine (20–40 μg) and oxotremorine (50–100 μg).

Picrotoxin also blocks the ganglionic depolarization produced by GABA (de Groat, 1970 ; de Groat, Lalley & Block, 1971). In the present experiments, when

tested against the depolarization elicited by similar doses of GABA (1–10 μg) and 5-HT (5–20 μg), picrotoxin (25–300 μg) appeared to be as effective as a GABA and 5-HT antagonist. On the other hand, bicuculline (50–300 μg), which is also a GABA-antagonist in the central nervous system (Curtis, Duggan, Felix & Johnston, 1971) and in peripheral ganglia (de Groat, *et al.*, 1971 ; de Groat, Lalley & Saum, 1972) did not modify the initial response to 5-HT but reversibly depressed the late response. Methysergide (100–500 μg) and competitive ganglionic blocking agents, hexamethonium (0·5 mg) and tetraethylammonium (50–300 μg), did not modify the responses to 5-HT. Atropine in doses (0·2–0·5 μg) sufficient to block muscarinic firing to cholinergic stimulant drugs (acetylcholine and methacholine) did not depress the early or late discharge to 5-HT. However, larger doses of atropine (5–20 μg) reduced the late response.

Cocaine (50–300 μg), an agent which is known to block the excitatory actions of 5-HT in ganglia (Trendelenburg, 1967), reduced the early depolarization and the early and late discharge elicited by 5-HT. Based on threshold blocking doses, cocaine was approximately equal in potency to picrotoxin as a 5-HT antagonist. However, cocaine was less selective than picrotoxin, since it reduced the responses to muscarinic ganglionic stimulants and reportedly blocks the excitatory actions of a variety of non-nicotinic ganglionic stimulating agents (Trendelenburg, 1967). Cocaine (50–300 μg) did not block the ganglionic depolarization evoked by GABA.

Bulbocapnine (50–300 μg) an agent which antagonizes the actions of 5-HT in various tissues (Walaszek & Chapman, 1963) blocked the ganglionic excitatory effects of 5-HT. However, the alkaloid also depressed the responses to acetylcholine and potassium chloride.

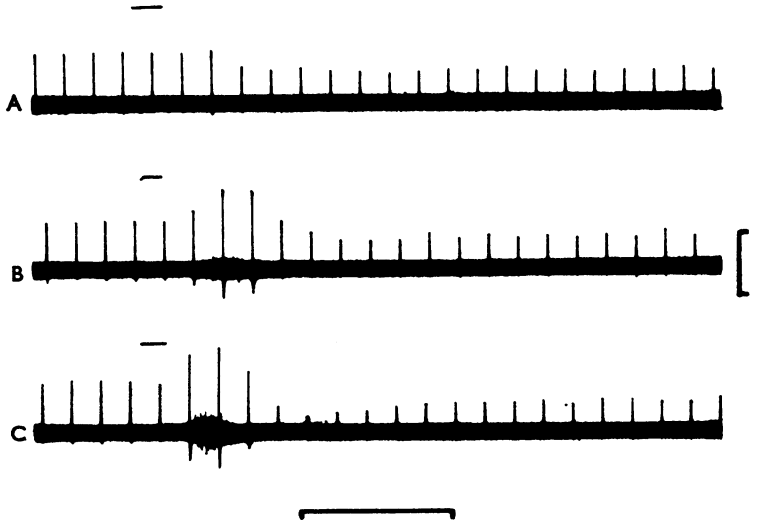

FIG. 6. Effect of graded doses of 5-hydroxytryptamine (5-HT) on transmission in the superior cervical ganglion. Recording on external carotid postganglionic branch. Records A, B and C, the responses evoked by 0·5, 2·5 and 5 μg of 5-HT, respectively. Postganglionic action potentials were elicited by submaximal stimulation of the preganglionic nerve at a frequency of 0·5 Hz. Action potentials were approximately 50% of maximal amplitude. Vertical calibration is 200 μV. Horizontal calibration is 10 seconds. Injections indicated by a bar above each record.

Effect of 5-hydroxytryptamine on ganglionic transmission

Other investigators have reported that 5-HT has a facilitatory effect on transmission in the superior cervical ganglion (Trendelenburg, 1956, 1957; Hertzler, 1961). In the present study this response was demonstrated in approximately 60% of the preparations (Figs. 6 and 7). Doses of 5-HT between 2–50 μg produced a transient (2–10 s) increase in the amplitude of postganglionic action potentials elicited by stimulation of the cervical sympathetic trunk (0·5–1 Hz) at submaximal intensities (approximately 50% of maximal). The enhancement of transmission was commonly less than 50% and was observed on both external carotid and 'spinal' postganglionic fibres. 5-HT also elicited a depression of transmission (Figures 5, 6 and 7). This response was observed in 28 of 33 experiments, on fibres to the external carotid artery and in 1 of 11 experiments on the 'spinal' nerves. Depression appeared at low doses of 5-HT (0·01–0·5 μg, threshold) and with very low doses occurred in the absence of a detectable facilitation of transmission (Figures 5 and 6). At higher doses of 5-HT the inhibition was usually preceded by facilitation (Figure 6). The magnitude of the depression ranged up to 70% reduction in spike amplitude with large doses of 5-HT (10–20 μg). The depression commenced after or on the decline of the ganglionic depolarizing potential (Fig. 5) and even with low doses of 5-HT was prolonged, lasting from 1 to 10 minutes. In approximately 25% of the experiments, the depression of transmission was associated with a hyperpolarizing ganglionic surface potential (Figure 5). In the remainder of the experiments the depression occurred during the late depolarization or when the demarcation potential had returned to control levels. Creatinine sulphate (50–100 μg) did not depress transmission.

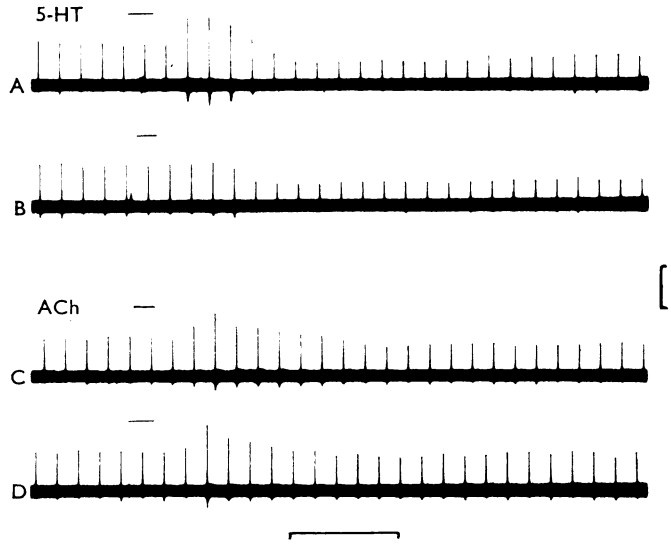

FIG. 7. Effect of 5-hydroxytryptamine (5-HT, 2·5 μg) and acetylcholine (ACh, 10 μg) on ganglionic transmission before and after the administration of picrotoxin (200 μg). Recordings on external carotid postganglionic branch. Records A and C, responses to 5-HT and ACh in the untreated ganglion. Records B and D, responses to 5-HT and ACh 1 min after the administration of picrotoxin. Postganglionic action potentials elicited by submaximal preganglionic nerve stimulation at a frequency of 0·5 Hz. Vertical calibration is 200 μV. Horizontal calibration is 10 seconds. Injections indicated by a bar above each record.

Picrotoxin in doses (50–200 μg) which depressed the 5-HT depolarizing potential also reduced the facilitation of transmission to 5-HT (Figure 7). The latter response however, was generally more resistant to picrotoxin than the 5-HT-evoked depolarization. Picrotoxin did not alter the 5-HT-induced depression of transmission or depression and facilitation elicited by injected acetylcholine (10–20 μg) (Figure 7). As reported previously (de Groat, 1970; de Groat *et al.*, 1971) picrotoxin in doses greater than 100 μg transiently depressed transmission. The interaction between picrotoxin and 5-HT was studied, therefore, after the direct depression to picrotoxin had dissipated. The facilitatory and depressant actions of 5-HT were not altered by atropine (0·5–6 μg), methysergide (200–300 μg) or bicuculline (50–250 μg).

We have also examined the effect of adrenergic blocking agents on the responses to 5-HT to determine whether the monoamine activated an adrenergic inhibitory mechanism in ganglia. Dihydroergotamine (50–100 μg) and bulbocapnine (200–400 μg) in doses which blocked adrenergic inhibitory mechanisms in pelvic ganglia (de Groat & Saum, 1971, 1972; Saum & de Groat, 1972a, b) and blocked the ganglionic depressant actions of noradrenaline (2–4 μg) did not modify the depression of transmission produced by 5-HT. However, bulbocapnine did block the facilitatory actions of 5-HT. Thus, it would appear that the depressant effects of 5-HT are not mediated by the intraganglionic release of catecholamines.

Depression by 5-hydroxytryptamine of muscarinic postganglionic firing

The ganglionic depressant effects of 5-HT were also demonstrated on the asynchronous firing elicited by injected muscarinic ganglionic stimulating agents. Large doses of 5-HT (5–20 μg) administered during the postganglionic discharge elicited by oxotremorine (50–100 μg, de Groat & Volle, 1963) produced an initial burst of firing followed by a reduction in the background discharge. Picrotoxin (100–200 μg) blocked the initial firing and also the late depression. Doses of 5-HT (0·5–2 μg) below the threshold for eliciting a discharge did not produce depression. Similar results were obtained when 5-HT was tested on the muscarinic firing elicited by an anticholinesterase agent (217AO, 75–150 μg) or by acetylcholine (10–30 μg). However, the nicotinic postganglionic discharge elicited by acetylcholine was not modified by the prior injection of a large dose of 5-HT (10–40 μg).

Discussion

The present experiments have revealed that picrotoxin is an effective antagonist of the ganglionic excitatory actions of 5-HT. Since picrotoxin did not block the responses to other ganglionic stimulating agents, it seems likely that its effects are mediated by a reversible blockade of 5-HT receptors. The ability of large doses of 5-HT to overcome the picrotoxin antagonism is consistent with this view. Other agents (e.g., cocaine and morphine) which block the ganglionic excitatory actions of 5-HT presumably act by different mechanisms (Trendelenburg, 1967), since they also depress the responses to a variety of non-nicotinic ganglionic stimulating agents.

The finding that picrotoxin is a 5-HT antagonist in ganglia raises the possibility that tryptaminergic mechanisms at other sites in the nervous system might be sensitive to the alkaloid. It seems reasonable therefore to inquire whether central

mechanisms, such as presynaptic inhibition, which are depressed by picrotoxin are mediated by 5-HT. The presynaptic inhibitory transmitter is thought to depolarize primary afferent terminals and thereby decrease the release of the excitatory transmitter (Eccles, 1964). Although various observations have indicated that GABA is the mediator of presynaptic inhibition (see Eccles, 1964 ; Levy, Repkin & Anderson, 1971 ; Davidson & Southwick, 1971 ; Davidoff, 1972 ; de Groat, 1972 ; de Groat *et al.*, 1972), there are data which would suggest that 5-HT might be involved as well. 5-HT is present in the mammalian brain and spinal cord and when administered exogenously is known to elicit either depressant or excitatory actions at various sites (Curtis & Crawford, 1969). In the lateral geniculate nucleus its depressant effects seem to be related in part to a presynaptic action (Curtis & Davis, 1962 ; Tebēcis & DiMaria, 1972). Exogenous 5-HT also depolarizes primary afferent terminals in the amphibian spinal cord (Tebēcis & Phillis, 1967), excites mammalian afferent fibres (Paintal, 1964 ; Jacobs & Comroe, 1971) and depolarizes the vagal sensory ganglion of the cat (de Groat, Lalley & Saum, unpublished). Thus, 5-HT has actions which one might expect of a presynaptic inhibitory transmitter. It will be important to determine in future experiments whether the depolarizing effect of 5-HT on afferent terminals is antagonized by picrotoxin.

It is evident from the present data that 5-HT produces depression as well as excitation in autonomic ganglia. The excitatory effects of 5-HT have been described previously by numerous investigators (see review by Trendelenburg, 1967) ; however, there are relatively few reports of ganglionic depression with 5-HT (Reinert, 1960 ; Jéquier, 1965 ; Machová & Bŏska, 1969). The depressant effects of 5-HT seem to vary in different ganglia and also with different pathways through the same ganglion. For example, in pelvic parasympathetic ganglia (Saum & de Groat, 1972c, d) 5-HT produced consistent and prominent depression ; the threshold dose for eliciting depression being 500 to 1,000 times less than the dose required to produce excitation. On the other hand, 5-HT elicited weak and variable depressant effects in inferior mesenteric ganglia (sympathetic) (Saum & de Groat, 1972c, d). The superior cervical ganglion seems to be even more complicated, since 5-HT depressed transmission in pathways to the external carotid postganglionic nerve ; but rarely depressed transmission to the ' spinal ' nerves. Thus, it would appear that ganglionic synapses vary considerably in their sensitivity to the actions of 5-HT. This may account, in part, for the failure of previous investigators to observe regularly depression with the monoamine.

Differences were also noted in the excitatory effects of 5-HT on external carotid and ' spinal ' pathways. In the unconditioned ganglion, 5-HT elicited an early discharge on both nerves ; however, following repetitive preganglionic stimulation a late discharge to 5-HT was unmasked on the ' spinal ' nerve, but only rarely on the external carotid nerve. It is interesting that in those pathways where 5-HT's depressant actions were prominent (i.e. pelvic ganglia and the external carotid pathway) the late discharge was absent and in pathways where 5-HT depression was weak (i.e. the ' spinal ' pathway) the late discharge was regularly observed.

The ganglionic depressant effects of 5-HT may be mediated by several mechanisms. Like nicotinic ganglionic stimulant drugs, 5-HT in large doses seems to elicit a postexcitatory depression and ganglionic hyperpolarization, which are probably associated with recovery processes in the ganglion cell (see Volle, 1966, and Haefely, 1972, for discussioins of the detailed mechanisms). 5-HT also seems

to have a direct depressant action on the ganglion since: (1) doses of 5-HT below the threshold for exciting ganglion cells could depress transmission and (2) picrotoxin antagonized the 5-HT excitation but did not modify the depression. It is not clear, however, whether the depression is related to a presynaptic or a postsynaptic action.

The physiological significance of the ganglionic excitatory and depressant actions of 5-HT is uncertain. There is no evidence that 5-HT is involved in transmission in the superior cervical ganglion or in other sympathetic ganglia ; however, 5-HT has been considered as a possible transmitter in the myenteric plexus of the stomach. Exogenous 5-HT has both excitatory and inhibitory actions in the gut and Bülbring & Gershon (1968) suggested that the latter may be of physiological importance. They proposed that 5-HT released from extrinsic tryptaminergic nerves excites intramural inhibitory neurones and these neurones in turn depress the smooth muscle of the gut. The inhibitory pathway, as conceived by Bülbring & Gershon, is consistent with the generally accepted view that 5-HT is a ganglionic stimulant. However, since the present experiments, and others in this laboratory (Saum & de Groat, 1972c, d) have revealed that 5-HT has prominent ganglionic depressant actions, there is the possibility that tryptaminergic pathways to the gut may also exert an inhibitory influence on neurones in the myenteric plexus. In future experiments the use of antagonists (e.g. picrotoxin) which selectively block the ganglionic excitatory responses to 5-HT, should allow one to distinguish between these two possibilities.

This investigation was supported in part by grant NB07923 from the National Institutes of Neurological Diseases and Stroke, and by a PHS Postdoctoral Fellowship to PML. W. C. de Groat is a recipient of a Research Career Development Award from NINDS. We thank Mr. J. Douglas, Mr. J. von Hedemann and Mr. T. Tokar for technical assistance.

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(*Received January* 9, 1973)