Br. J. Pharmac. (1975), 55, 27-40

THE ACTION OF 5-HYDROXYTRYPTAMINE ON CHEMORECEPTOR DISCHARGES OF THE CAT'S CAROTID BODY

K. NISHI

Department of Pharmacology, Kumamoto University Medical School, Honjo 2-2-1, Kumamoto, Japan

1 Chemoreceptor discharges were recorded *in vivo* from fine filaments of the carotid sinus nerve containing a single or several active units; their frequency was used as an index of receptor activity. The effects of 5-hydroxytryptamine (5-HT) on chemoreceptors were studied in 26 adult cats. At times, sinus baroreceptor discharges were recorded from the carotid nerve and the effect of 5-HT on the discharges was examined.

2 Intra-carotid injections of 5-HT (2-20 μg) induced a sharp and brief increase in chemoreceptor discharges, followed by depression or block which lasted for several seconds. Repeated injections at short intervals, and a small dose after a large dose of 5-HT resulted in depressed or blocked response to 5-HT.

3 5-HT in high doses (10-20 μg, i.a.) slightly depressed the chemoreceptor discharges induced by either acetylcholine (ACh) or NaCN, when these substances were applied within 20 s after 5-HT. 5-HT (5-20 μg, i.a.) applied during asphyxia induced a further increase in chemoreceptor discharges, soon followed by block of the discharges lasting for several seconds.

4 Atropine or hexamethonium in high doses did not change the chemoreceptor response to 5-HT, while that to ACh was markedly depressed.

5 (+)-Lysergic diethylamide (LSD), methysergide or gramine did not alter the response to 5-HT, while LSD in low doses produced a marked increase in chemoreceptor discharges..

6 Acute and chronic treatment with reserpine (5-10 mg/kg, i.v.) of the animals did not change the sensitivity and the reactivity of the chemoreceptor to ACh and NaCN, while the chemoreceptor response to 5-HT was augmented, indicating an increase in the sensitivity of chemoreceptors to 5-HT.

7 5-HT in small doses (2-10 μg, i.a.) induced a marked increase in sinus baroreceptor discharges; subsequently discharges were depressed or blocked for several seconds.

8 The results are discussed in relation to possible mechanism of action of 5-HT on the chemoreceptors. It is concluded that the exogenous 5-HT probably acts directly on the chemosensory nerve endings and depolarizes them, but 5-HT contained in the carotid body does not play a significant role in the generation of chemoreceptor discharges.

Introduction

Catecholamines and indolamine are known to be normally present in the carotid body of different mammalian species (Lever, Lewis & Boyd, 1959; Niemi & Ojala, 1964; Fillenz & Woods, 1966; Hamberger, Ritzen & Wersäll, 1966; Chiocchio, Biscardi & Tramezzani, 1966, 1967; I-Li Chen, Yates & Duncan, 1967; Zapata, Hess, Bliss & Eyzaguirre, 1969; Kobayashi, 1971; Chiocchio, King, Carballo & Angelakos, 1971). Whether they play a role in initiating or modulating chemoreceptor activity of the carotid body is still not clear. Some authors have indicated that catecholamines may not have a significant role in chemoreceptor function (Eyzaguirre & Koyano, 1965; Eyzaguirre & Zapata, 1968; Zapata *et al.*, 1969), although other evidence indicates otherwise (Biscoe, 1965; Sampson, 1972; Andrew, Black, Comroe & Jacobs, 1972). A physiological role for 5-hydroxytryptamine (5-HT) may not be discarded, since the carotid body contains a certain amount of 5-HT (Chiocchio *et al.*, 1967, 1971); furthermore, histochemical methods have shown that 5-HT is located in the electron-dense core vesicles of the Type I cells (Chiocchio *et al.*,

1967; I-Li Chen *et al.*, 1967). These findings lend some support to the view that 5-HT may have some functional role in the carotid body. However, reports on the effects of 5-HT on carotid body chemoreceptors have been contradictory. Some authors have reported pronounced chemoreceptor stimulation by 5-HT (Douglas & Toh, 1952; Ginzel & Kottegoda, 1954; McCubbin, Green, Salmoiraghi & Page, 1956; Comroe & Mortimer, 1964), while others have failed to observe such an effect (cf. Heymans & Neil, 1958; Eyzaguirre & Koyano, 1965). More recently, Andrew *et al.* (1972) have shown a species difference in the response of chemoreceptors to 5-HT; the agent stimulates carotid body chemoreceptors and produces reflex hyperpnoea in dogs, but not in cats.

In most experiments done by previous investigators, reflex changes in respiration were used as indicators of chemoreceptor activity of the carotid body. However, changes in respiration after 5-HT do not seem to be the most sensitive indicator of chemoreceptor activity, since these effects may be complicated by other neurogenic or circulatory influences (cf. Comroe, 1966; Black & Torrance, 1971; Jacobs & Comroe, 1971).

The present experiments were designed to examine the direct action of 5-HT on carotid body chemoreceptor discharges in an attempt to clarify the discrepancies previously reported.

Methods

The methods employed in the present experiments were essentially similar to those described in a previous paper (Nishi & Eyzaguirre, 1971).

Twenty-six adult cats were anaesthetized with sodium pentobarbitone (40 mg/kg, i.p.). The trachea was cannulated for artificial ventilation. A positive pressure respirator (Harvard Apparatus, 660) was set to provide an arterial P_{O_2} of approximately 90 mmHg, and a P_{CO_2} of 40 mmHg, and both the P_{O_2} and P_{CO_2} were periodically measured during the experiment, using Astrup micro-equipment (Radiometer D616). To maintain constant ventilation throughout an experiment, pneumothorax was made by opening a hole in the thoracic cavity. Both vago-sympathetic trunks and the left carotid sinus nerve were sectioned to prevent respiratory reflex movements arising from either injection of chemicals or asphyxia (Heymans & Neil, 1958). The right carotid nerve was exposed and cut at its junction with the glossopharyngeal nerve. The nerve was cleaned of surrounding connective tissue, and prepared for recording. Baroreceptor activity was eliminated by cutting or crushing the nerve branches going to the sinus region. In a few cases, a filament containing baroreceptor activity was selected to examine the effects of injected chemical substances on impulse conduction. Sympathetic nerves going to the carotid sinus region were severed to avoid possible efferent effects.

The peripheral end of the carotid nerve was dissected into fine filaments containing either a single or a few active units. Nerve impulses were led off from the nerve with platinum bipolar electrodes, amplified for display on an oscilloscope (Tektronix 5103N), and simultaneously led to an FM-Data recorder (Sony PFM-5). The nerve impulses were led to an impulse height discriminator, whose output pulses were fed to a counter-D-A converter system (Yokokawa, Hewlett & Packard 5302A, 5311A). Nerve impulses were counted for 0.2 or 1 s periods at 100 μs intervals. Arterial blood pressure was recorded from the femoral artery by an electronic manometer (Nihonkoden MT-4). Its output and that of the D-A converter were displayed on an ink-writing recorder, while impulses were monitored on a cathode-ray oscilloscope. The nerve impulses and pressure records were registered on different channels of the data-recorder for subsequent analysis.

A small polyethylene catheter was inserted into the right common carotid artery through a muscular branch of the artery, the tip of the catheter being placed about 2 mm caudal to the origin of the superior thyroid artery. Chemical substances were injected into the common carotid artery through the catheter, or into the femoral vein. In the case of an intra-arterial injection, all chemicals were dissolved in Locke solution (pH 7.3) at 34°-35° C; the volume of the injected solution was 0.25 ml. The doses used are expressed as the salt. The substances employed were: acetylcholine chloride (Daiichi Seiyaku), sodium cyanide (Merck), 5-hydroxytryptamine creatinine sulphate complex (Wako Pure Chemicals), creatinine sulphate (Wako Pure Chemicals), (−)-methyl-(+)-lysergic acid butanolamide (methysergide) (Sandoz), (+)-lysergic acid diethylamide (LSD) (Sandoz), gramine hydrochloride (Wako Pure Chemicals), atropine sulphate (Merck) and reserpine (Boehringer-Mannheim). All experiments were conducted at room temperatures of 20°-25° C.

Results

Chemoreceptor discharges were identified by: (1) making intra-arterial injections of a small amount of NaCN (2 μg), which increased the discharge

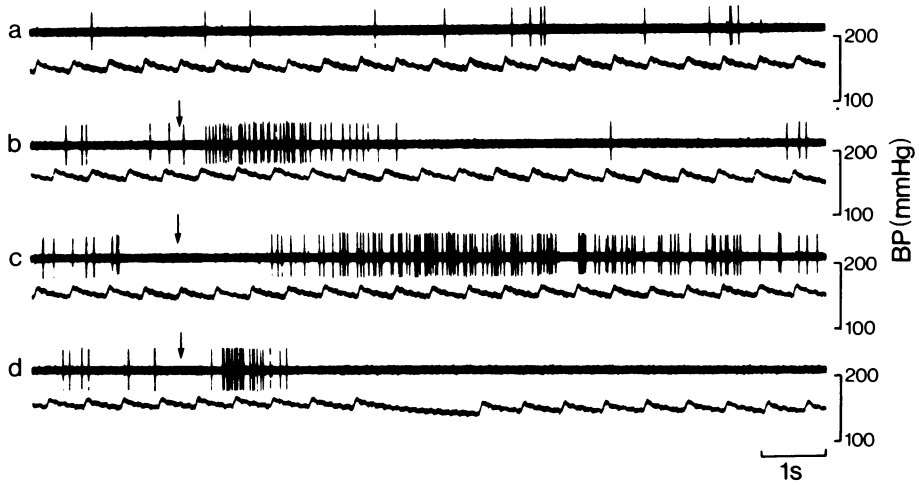

Figure 1 Effect of intra-arterial injections of acetylcholine (ACh), NaCN and 5-hydroxytryptamine (5-HT) on single chemoreceptor fibre discharge in the cat. Upper traces show impulse activity and lower ones, arterial blood pressure. The animal was artificially ventilated with room air and the resting rate of the discharge of the unit was 2-4 s at an arterial PO_2 of 94 mmHg and a PCO_2 of 41 mmHg (a). ACh (2 μg in 0.25 ml Locke solution) (b), and NaCN (2 μg in 0.25 ml Locke solution) (c) elicited a considerable increase in chemoreceptor discharges. In (d) 5-HT (2 μg in 0.25 ml Locke solution) was given. Injections were made at arrows.

frequency 2-3 s after the injections (Nishi & Eyzaguirre, 1971), (2) ventilating the animal with pure O_2, which caused a decrease or abolition of spontaneously appearing discharges.

In animals ventilated with room air, the resting discharge frequency of single units ranged from 0-12 impulses/s at an arterial PO_2 of 85-98 mmHg. ACh or NaCN, given intra-arterially, evoked threshold responses in doses of 0.5-2 μg; the response to ACh was transient and disappeared within 5 s, while NaCN produced a gradual increase in chemoreceptor discharges.

The action of 5-hydroxytryptamine on the chemoreceptor discharge

In all cases 5-HT was injected intra-arterially and caused a fall in blood pressure. This effect appeared after a latent period of 5-7 s from the start of the injection and lasted for 10-20 seconds. The fall in blood pressure following the intra-arterial injection of 5-HT was depressed or abolished, after the nodose ganglion was excised. The results are consistent with those obtained by Andrew *et al.* (1972). The depressor response is most probably due to excitatory effects of 5-HT on the nodose ganglion, which in turn, produces depressor reflexes (Jacobs & Comroe, 1971). The records of Figure 1 illustrate a typical unitary response to ACh, NaCN and 5-HT. Each substance elicited a marked increase in chemoreceptor discharges. When 5-HT (2 μg) was delivered through the artery, the discharges abruptly increased with a latency of about 0.5 s (measured from the start of the injection to the onset of discharges) (Figure 1d). This excitatory effect of 5-HT was transient, and disappeared within 2 s; it was followed by depression of the spontaneously occurring discharges. Thereafter, the discharge frequency gradually returned to the control level. In most instances, the response of chemoreceptor discharges to intra-arterial injections of 5-HT consisted of two phases; an initial transient increase in discharge frequency followed by a decrease or complete block of the spontaneously occurring discharges. Occasionally, during the course of an experiment, some units showed a change in their response pattern to 5-HT; after a decrease in the discharge frequency, the discharges again started to increase gradually, returning to the control level within 10 seconds.

When a given dose of 5-HT was injected a number of times within a short period, the response to the second dose varied depending on the intervals at which the injections were delivered. In most cases, the response to the second dose was greatly reduced, and after an initial larger dose, a previously effective smaller dose was without effect for a longer period (Figure 2). The first injection of 5-HT (2 μg, i.a.) induced a sharp transient increase in discharges, while the second one delivered 6 s later did not elicit a

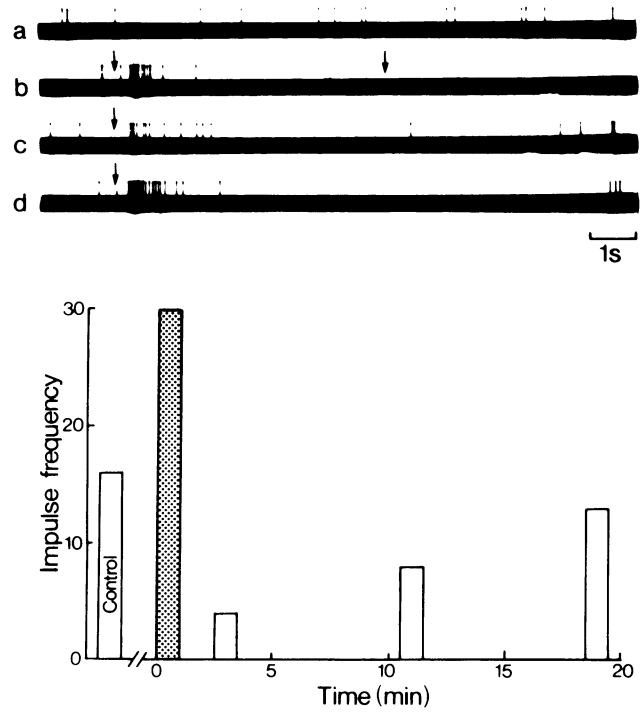

Figure 2 Effect of repeated intra-arterial injections of 5-hydroxytryptamine (5-HT) on chemoreceptor activity; (a)-(d) show effects of repeated injections of 5-HT on single chemoreceptor fibre discharges. (a) Spontaneous discharge during control period; (b) 5-HT (2 μg in 0.25 ml Locke solution) applied successively at arrows; (c) 5-HT (2 μg) injected 5 min after the second injection of 5-HT in (b); (d) 5-HT (2 μg) 15 min after 5-HT in (c). Injections were made at arrows.

Lower graph illustrates effects of a large dose of 5-HT on subsequent chemoreceptor responses to a small dose of 5-HT. Open bars represent the number of chemoreceptor discharges in a few active units-preparation induced by 5-HT (2 μg in 0.25 ml Locke solution), and the dotted bar that induced by 5-HT (20 μg in 0.25 ml Locke solution). 5-HT (20 μg) was applied 30 min after the control 5-HT.

response (Figure 2b). The third injection. 5 min after the second one, produced a slight increase in the discharge (Figure 2c), while spacing the 5-HT deliveries at longer intervals (15 min in Figure 2d) produced a response similar to that obtained during the control period. The effect of a large dose of 5-HT on the responses to small doses subsequently applied, are illustrated in the lower graph of Figure 2, where the discharge frequency from a few units preparation was counted during 5 s after the start of the injections of 5-HT. The depressant effect of the large dose of 5-HT on the responses to smaller doses lasted for about 30 minutes.

The relationship of the two components of the biphasic response to 5-HT varied with different doses of the agent. On increasing the doses to 20 μg, the number of chemoreceptor discharges occurring within a few seconds after the injections, increased, but the dose-response curve appears S-shaped, while the period during which spontaneous discharges were blocked or depressed was prolonged proportionally to the dose of 5-HT. A quantitative estimate of effects of increasing doses of 5-HT on chemoreceptor discharges is shown in Figure 3, where the upper records were taken from discharges of a single chemoreceptor unit responding to different doses of 5-HT; in the lower graph dose-response curves were constructed from discharges of a few active unit preparations.

Removal of superior cervical ganglion 5-HT in small doses (0.5-5 μg) injected into the common carotid artery stimulates the superior cervical ganglion in the cat (Trendelenburg, 1958, 1959; Jaramillo & Volle, 1968). Stimulation of the sympathetic ganglia by 5-HT could release catecholamines from the sympathetic nerve endings in the carotid body, which in turn, could cause a decrease in blood flow through the carotid

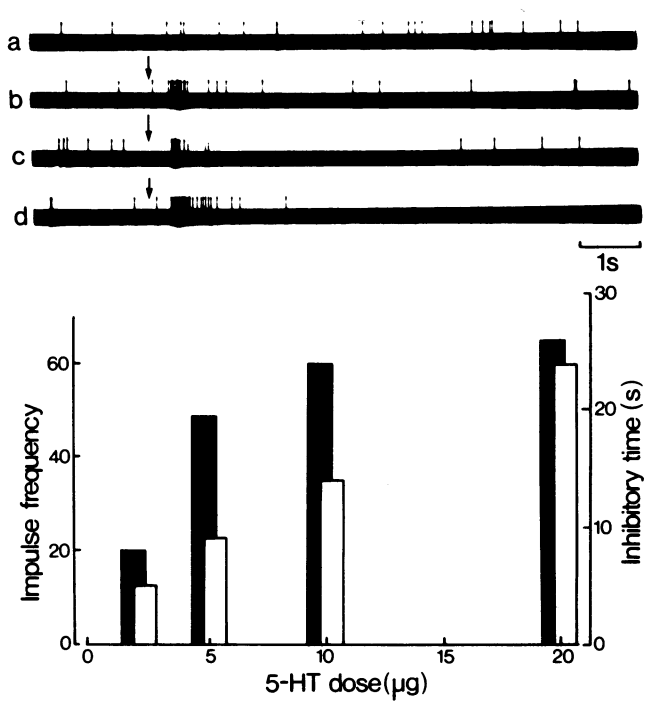

Figure 3 Chemoreceptor responses evoked by different doses of 5-hydroxytryptamine (5-HT); (a)-(d) show effects of intra-arterial injections of different doses of 5-HT on single chemoreceptor fibre discharges. (a) Spontaneous discharge in control period; (b) 5-HT (2 μg in 0.25 ml Locke solution); (c) 10 μg; (d) 20 μg. Injections were made at arrows every 20 minutes. Lower graph illustrates dose-response curves to 5-HT. Since there was tachyphylaxis to 5-HT, different doses of 5-HT were given every 20 minutes. The total number of chemoreceptor discharges of a few active units was counted during 5 s after the injections, and is shown by black columns, while the time during which the discharge frequency decreased below the control level after 5-HT is indicated by open columns.

body. This could lead to increased chemoreceptor activity. Furthermore, dopamine, which may be released from the sympathetic nerve endings (Thoenon, Haefely & Gey, 1967) could act directly on chemoreceptors and cause a decrease in chemoreceptor discharges, since this agent has been reported to decrease or block chemoreceptor discharges from the carotid body in the cat (Sampson, 1972). If this was the case, the present results could have been due primarily to a stimulating action of 5-HT on the superior cervical ganglion.

To clarify this possibility the effects of 5-HT on chemoreceptor discharges were examined after the right superior cervical ganglion had been removed. 5-HT injected intra-arterially induced a change in chemoreceptor discharges, which was identical to that obtained in the animals with intact superior cervical ganglia. Thus, the action of 5-HT on chemoreceptor discharges is not due to secondary effects mediated through sympathetic stimulation.

Effects of 5-hydroxytryptamine antagonists Since 5-HT may affect the local carotid body circulation by either a constrictor or dilator effect which could affect chemoreceptor activity, methysergide, LSD and gramine hydrochloride which are very potent antagonists of the effects of 5-HT on peripheral and muscular receptors of the agent (see Gyermek, 1966), were employed to prevent possible effects of 5-HT on the vessels of the carotid body.

Effects of LSD and methysergide on the responses of chemoreceptor discharges to 5-HT are shown in Figure 4. The upper records (a)-(c) were taken from a several-units preparation and the lower ones were obtained from a different animal. An intra-arterial injection of 5-HT produced a transient increase in discharge frequency and later abolished the spontaneous discharges (Figure 4a). LSD (10 μg, i.a.) delivered 20 min after the previous injection of 5-HT, elicited a marked increase in the discharges (Figure 4b). An

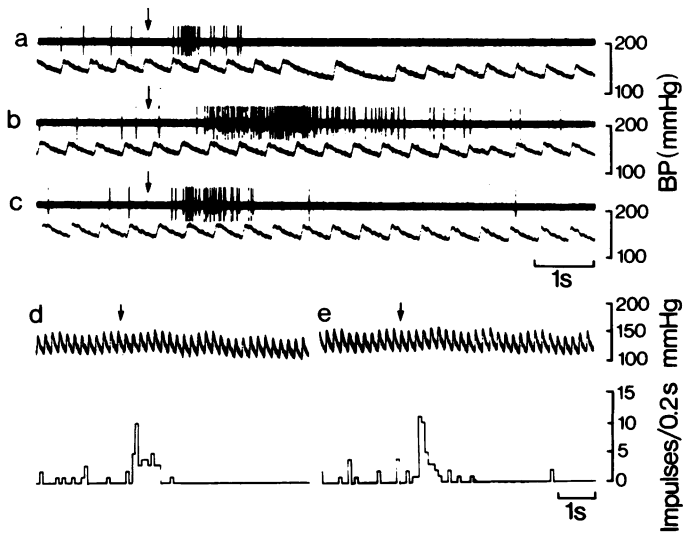

Figure 4 Effect of (+)-lysergic acid diethylamide (LSD) and (−)-methyl-(+)-lysergic acid butanolamide (UML) on chemoreceptor response to 5-hydroxytryptamine (5-HT). (a)-(c) Show effects of 5-HT on single chemosensory fibre discharges before and after LSD. Injections made at arrows. (a) 5-HT (5 μg in 0.25 ml Locke solution, i.a.); (b) LSD (10 μg in 0.25 ml Locke solution, i.a.); (c) 5-HT (5 μg in 0.25 ml Locke solution, i.a.) 5 min after LSD (100 μg/kg, i.v.). Tracings in (a)-(c) as in Figure 1. See text. (d)-(e) Show chemoreceptor responses to 5-HT before and after UML. Upper tracings show arterial blood pressure changes and lower ones, discharge frequency in a few active chemoreceptor units. Injections made at arrows. (d) 5-HT (5 μg in 0.25 ml Locke solution, i.a.) applied 30 min before UML; (e) 5-HT (5 μg in 0.25 ml Locke solution, i.a.) applied 5 min after UML (100 μg/kg, i.v.).

intravenous injection of LSD (100 μg/kg) after the previous injection in (b) also caused a gradual increase in the discharge frequency (not illustrated). Shortly after the intravenous injection of the agent, 5-HT was delivered. The response of chemoreceptor discharges to 5-HT was practically identical to that shown in the control, while the change in blood pressure caused by 5-HT was prevented by LSD (Figure 4c). LSD in doses up to 100 μg/kg did not affect the excitatory and inhibitory components of the response of chemoreceptors to 5-HT.

Intra-arterial or intravenous injections of methysergide (10-100 μg, i.a., 50-100 μg/kg, i.v.), and gramine (50 μg, i.a., 50 μg/kg, i.v.) did not produce any change in the frequency of spontaneous chemoreceptor discharges. The responses of chemoreceptor discharges to 5-HT before and after methysergide (100 μg/kg, i.v.) are shown in Figure 4d and e. Neither methysergide nor gramine altered the response to 5-HT.

The experiments just described are not conclusive with regard to possible vascular effects of 5-HT in the carotid body. However, they indicate that the effects of 5-HT on chemoreceptor discharges are probably due to a direct action on mechanisms responsible for the initiation of chemoreceptor discharges.

Effects of atropine and hexamethonium It has been proposed that ACh plays an important role in the generation of chemoreceptor discharges in the carotid body (Eyzaguirre & Zapata, 1968; Eyzaguirre, Nishi & Fidone, 1972). If this is also the case with regard to 5-HT, there is a possibility that the action of 5-HT on chemoreceptors may be mediated through a cholinergic mechanism. Atropine and hexamethonium were used to examine effects of cholinergic blocking agents on 5-HT responses.

Since large doses of atropine or hexamethonium can block or depress the responses to ACh and NaCN, when these agents are applied shortly after the cholinergic blocking agents (Nishi & Eyzaguirre, 1971), the same doses of cholinergic blocking agents were employed in the present experiments. Shortly after intravenous injections of either atropine (2-5 mg/kg) or hexamethonium (10-30 mg/kg), a small dose of 5-HT was injected intra-arterially (Figure 5). Intra-arterial injections of ACh and 5-HT produced a transient increase in the discharges during the control period (Figure

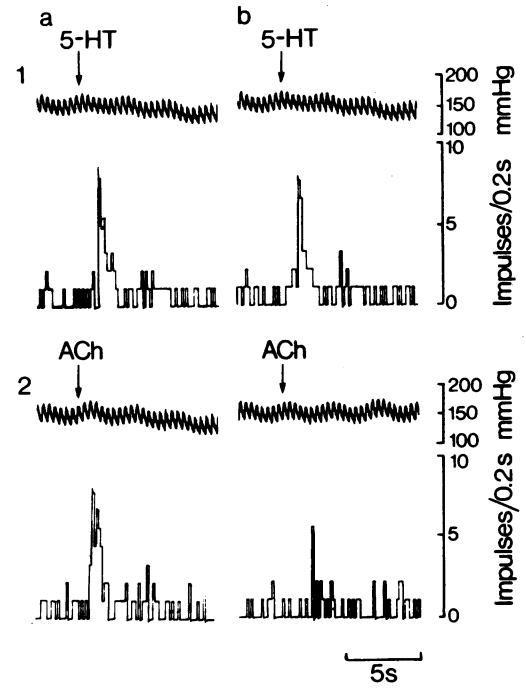

Figure 5 Effect of atropine on chemoreceptor response to 5-hydroxytryptamine (5-HT) and acetylcholine (ACh); (a) before; (b) 5-7 min after atropine (5 mg/kg, i.v.). Upper traces show arterial blood pressure and lower traces chemoreceptor discharge frequency in a few active units. Injections made at arrows. (a1) and (b1), 5-HT (2 μg in 0.25 ml Locke solution, i.a.); (a2) and (b2), ACh (5 μg in 0.25 ml Locke solution, i.a.).

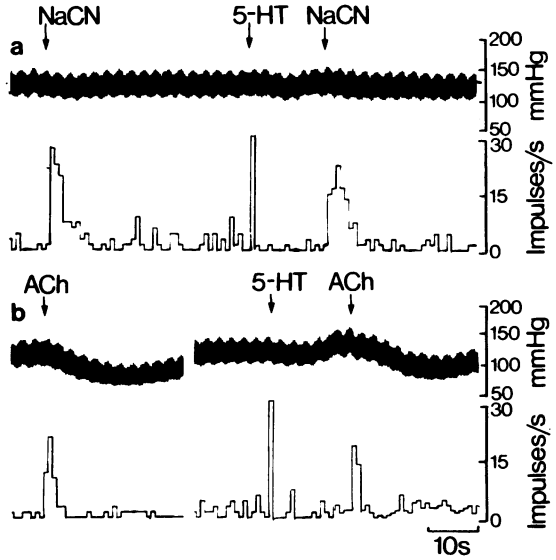

Figure 6 Effect of 5-hydroxytryptamine (5-HT) on chemoreceptor response to acetylcholine (ACh) and NaCN. Upper traces show arterial blood pressure changes. Lower traces, discharge frequency in a few active chemoreceptor units. (a) NaCN (2 μg in 0.25 ml Locke solution, i.a.) and 5-HT (20 μg in 0.25 ml Locke solution, i.a.) injected at arrows; (b) ACh (2 μg in 0.25 ml Locke solution, i.a.) and 5-HT (20 μg in 0.25 ml Locke solution, i.a.) injected at arrows.

5a). Five minutes after atropine (5 mg/kg, i.v.), the same doses of ACh and 5-HT used in the control were given. The response to 5-HT remained unchanged, while that to ACh was greatly depressed (Figure 5b).

The results are consistent with earlier observations *in vivo* where in the presence of atropine or hexamethonium the reflex response of respiration to 5-HT remained unaltered, while neither nicotine nor ACh caused any change in respiration (Ginzel & Kottegoda, 1954). Thus, it may be concluded that a cholinergic mechanism is not involved in eliciting the response of chemoreceptor discharges to 5-HT.

Creatinine sulphate Krnjević (1965) has noted that creatinine has a depressant action on some cortical neurones, while in other regions, e.g. the brain stem, the agent is mainly an excitant (Bradley & Wolstencroft, 1965). Thus it was possible that the creatinine moiety contained in 5-HT creatinine sulphate complex employed in the present experiments could have participated in producing the responses to 5-HT. To study this possibility, the effects of creatinine sulphate on chemoreceptor discharges were examined in four different animals.

In all cases creatinine sulphate (0.5-20 μg, i.a.) did not produce any change in chemoreceptor discharge frequency, while a slight fall in blood pressure was observed after injections of relatively large doses of the agent, indicating that the response to 5-HT obtained in the present experiments can be attributed only to the 5-HT moiety.

Effects of 5-hydroxytryptamine on the responses to acetylcholine, NaCN and asphyxia

The effects of 5-HT on the responses of chemoreceptor discharges to ACh and NaCN were examined in five different cats. ACh and NaCN injected intra-arterially produced a marked increase in chemoreceptor discharges. After the discharge frequency returned to the control level, 5-HT (20 μg, i.a.) was injected. Shortly after the injection of 5-HT, ACh and NaCN were injected

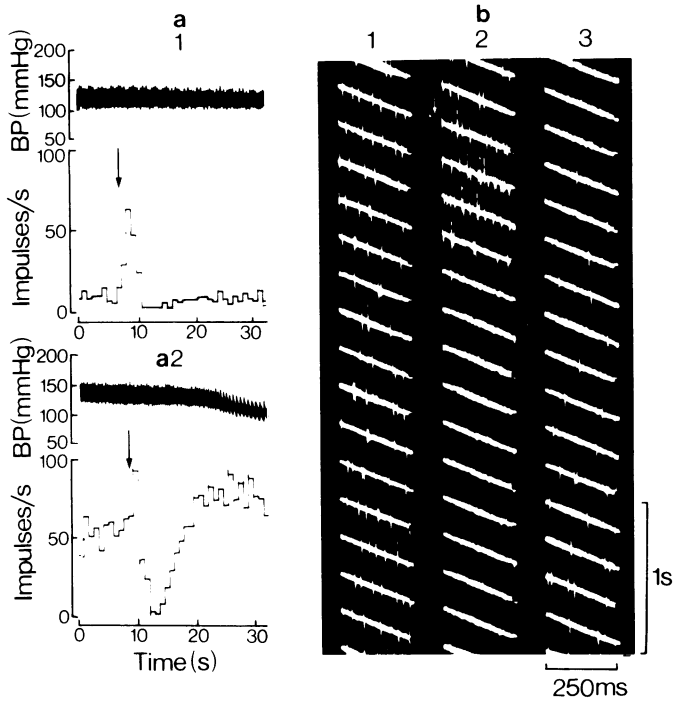

Figure 7 Effect of 5-hydroxytryptamine (5-HT) on chemoreceptor discharge during asphyxia (a1), 5-HT (2 μg in 0.25 ml Locke solution, i.a.) in control period, and (a2), same dose of 5-HT during asphyxia. Upper traces show arterial blood pressure changes. Lower traces, discharge frequency in a few active chemoreceptor units. Injections made at arrows. (b) Effect of 5-HT (5 μg in 0.25 ml Locke solution, i.a.) on single chemosensory fibre discharges during asphyxia: (b1, 2 and 3) continuous records. Injection made at arrow. Records read from top to bottom and left to right.

repeatedly every 5 minutes. The response to ACh or NaCN was slightly depressed when these substances were applied within 20 s after the injection of 5-HT. Otherwise, the response to ACh remained unchanged and subsequent injections of NaCN produced responses similar to control ones. A slight depression of the responses to ACh and NaCN observed immediately after 5-HT was relieved by larger doses of the agents. An example is illustrated in Figure 6. In Figure 6a, NaCN (2 μg) was injected intra-arterially 15 s after 5-HT (20 μg, i.a.), the response to NaCN being slightly depressed. Thirty minutes after the injection of 5-HT in Figure 6a, the same dose of 5-HT evoked a response similar to the previous one and ACh (2 μg), injected 17 s after the 5-HT, produced a response practically identical to that obtained during the control period (Figure 6b).

Figure 7 shows the effects of 5-HT on chemoreceptor discharges during asphyxia produced by stopping the artificial respirator in two different animals. During the control period, 5-HT (2 μg, i.a.) induced a marked increase in discharges followed by depression which lasted for about 8 s (Figure 7a(1)). One minute after the onset of asphyxia, when the discharges attained their maximal frequency, 5-HT (2 μg, i.a.) produced a further increase in discharges for a short period and thereafter, the discharge was depressed for about 10 s (Figure 7a(2)). Actual records obtained from a single-unit preparation in another cat also show both excitatory and inhibitory effects of 5-HT (5 μg, i.a.) on chemoreceptor discharges during asphyxia (Figure 7b(1), (2) and (3)). The inhibitory action of 5-HT on the discharges lasted for 10 s, the discharge frequency returning to the level before the 5-HT injection.

Thus, 5-HT had a transient inhibitory action on chemoreceptor responses to ACh, NaCN and asphyxia.

Effects of reserpine on responses to 5-hydroxytryptamine, acetylcholine and NaCN

Zapata *et al.* (1969) have shown that acute and chronic reserpine-treatment of cats does not

change the sensitivity and reactivity of the carotid body to ACh, anoxic or asphyxic stimulation. Their results could suggest that 5-HT is not acting as a main 'excitatory transmitter substance' in the carotid body, since reserpine depletes 5-HT in various tissues (see Carlsson, 1966) and may have reduced the 5-HT content in the carotid body. However, 5-HT contained in the carotid body, may play a more subtle role in modulating a mechanism responsible for the initiation of chemoreceptor discharges. If endogenous 5-HT is acting on this mechanism, reserpine-treatment would be expected to increase the sensitivity to exogenous 5-HT in the carotid body, as reserpine sensitizes other preparations to catecholamines (Bein, 1956; Burn & Rand, 1958; Axelrod, 1964). Therefore, it was interesting to see whether reserpine changed the responses to 5-HT, ACh and NaCN.

In five cats, two or three nerve filaments containing a few active chemoreceptor units were selected and prepared for recording. In all cases, the right superior cervical ganglion was removed to prevent possible vascular effects on the circulation within the carotid body. After testing the sensitivity of chemoreceptor units to 5-HT, ACh and NaCN, one nerve filament was retained on the electrodes for continuous recording. Thereafter, reserpine (5-10 mg/kg) was injected intravenously and the responses of chemoreceptor units to 5-HT, ACh and NaCN were periodically examined for 20 hours. Some chemoreceptor units stopped discharging during the course of an experiment, probably because of deterioration of the nerve fibre in the filament. In such cases, the nerve filaments were discarded. Because of difficulty in obtaining a stable record for a long period, only five chemoreceptor units in two nerve filaments from two different cats survived for 20 hours.

Figure 8 illustrates an experiment in which chemoreceptor discharges of two units were recorded before and after reserpine. During the control period, the resting rate of discharges of each unit was 0-8 impulses/s and 2-5 impulses/s at an arterial Po_2 of 92 mmHg and a mean arterial blood pressure of 130 mmHg (Figure 8a). Immediately after an intravenous injection of reserpine (10 mg/kg), the spontaneous discharges disappeared, concomitant with a marked fall in blood pressure, and responses to ACh, NaCN and 5-HT were markedly depressed. The spontaneous discharges reappeared within 10 min, but the discharge frequency was low (Figure 8b). The depression of chemoreceptor discharges lasted for another 60 minutes. Thereafter, the discharge frequency gradually increased. Four hours after reserpine, the resting rate of the discharges was 5-12 impulses/s and 6-9 impulses/s at a Po_2 of

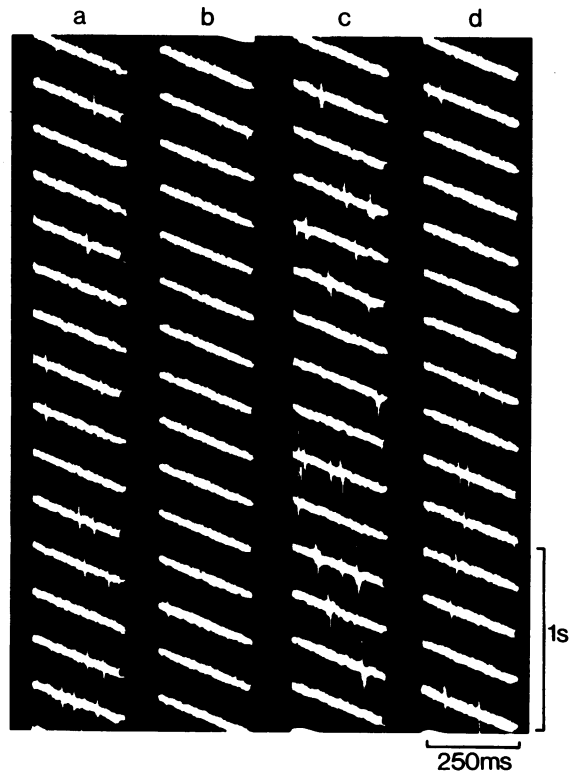

Figure 8 Effect of reserpine on chemoreceptor activity. Records (a)-(d) taken from a same filament containing two active chemoreceptor units. (a) Spontaneous discharge in control period; (b) 10 min after reserpine (10 mg/kg, i.v.); (c) 4 h after reserpine; (d) 20 h after reserpine.

88 mmHg and a mean blood pressure of 98 mmHg (Figure 8c). ACh, NaCN and 5-HT induced pronounced responses of chemoreceptor discharges. Twenty hours after reserpine, blood pressure was still low (mean arterial pressure; 105 mmHg), but the resting rate of the discharges slightly decreased, compared with that observed 4-5 h after reserpine (Po_2; 87 mmHg) (Figure 8d). ACh and NaCN increased the chemoreceptor discharges, the response patterns to both chemicals being essentially similar to those obtained during the control period, while 5-HT inhibited the spontaneously appearing discharge after exerting a short excitatory effect.

Figure 9 illustrates another experiment in which chemoreceptor responses to 5-HT, NaCN and ACh were tested before and after reserpine. Control responses of three chemoreceptor units to 5-HT (2 μg, i.a.), NaCN (2 μg, i.a.) and ACh (5 μg, i.a.) are shown in column (a). Four hours after reserpine (5 mg/kg, i.v.), the responses to same doses of ACh and 5-HT used in the control period

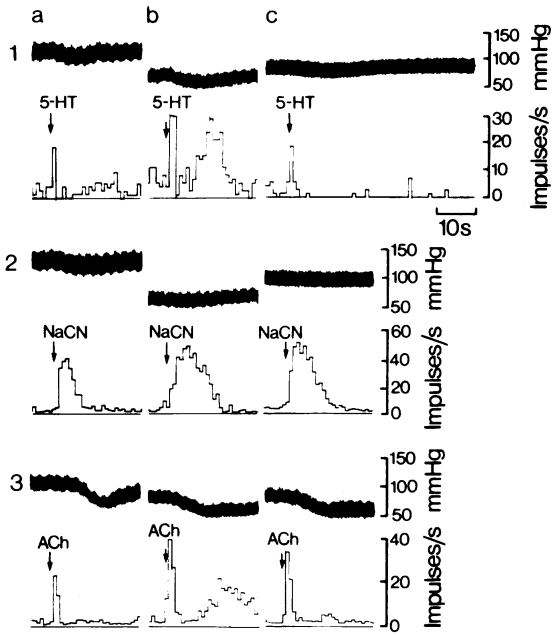

Figure 9 Chemoreceptor response to 5-hydroxytryptamine (5-HT), NaCN and acetylcholine (ACh) before and after reserpine. Upper traces show arterial blood pressure changes. Lower traces, discharge frequency in a few active chemoreceptor units. Injections made at arrows. (a) Control; (b) 4 h after reserpine (5 mg/kg, i.v.); (c) 20 h after reserpine. (1) 5-HT (2 μg in 0.25 ml Locke solution, i.a.); (2) NaCN (2 μg in 0.25 ml Locke solution, i.a.); (3) ACh (5 μg in 0.25 ml Locke solution, i.a.). Records obtained from the same filament containing three chemoreceptor units before and after reserpine.

were very pronounced and NaCN also produced a long-lasting chemoreceptor stimulation (Figure 9b); this effect was probably influenced by changes in local circulation of the carotid body; because of the marked hypotension and bradycardia, the chemical substances may have reached and left slowly the sites responsible for initiating chemoreceptor discharges. Twenty hours after reserpine, both NaCN and ACh produced responses similar to those in the control, while 5-HT depressed the discharges for about 40 s, after inducing a sharp and transient increase in the discharges (Figure 9c). It is important to notice that the inhibitory phase of the response to 5-HT after reserpine was markedly prolonged. Furthermore, a previously ineffective dose of 5-HT (0.5 μg, i.a.) produced a response, a sharp initial increase and subsequent depression of chemoreceptor discharges.

The experiments just described show pronounced effects of ACh, NaCN and 5-HT on chemoreceptor activity after prolonged reserpine-treatment. Qualitatively, there was no appreciable difference between chemoreceptor activation induced by ACh and NaCN before and after reserpine. The results are consistent with those obtained by Zapata *et al.* (1969). However, it is important to notice that the response pattern to 5-HT changed after reserpine, the inhibitory phase of the response being greatly prolonged. This seems to indicate that chemoreceptors may become more sensitive to 5-HT after reserpine.

Effects of 5-hydroxytryptamine on baroreceptor discharges

In order to compare the effects of 5-HT on chemoreceptor discharges with those on other sensory discharges, the baroreceptor discharges frequency was examined after intra-arterial injections of various doses of 5-HT. Nerve filaments containing a single or several units of baroreceptor activity were selected from the carotid sinus nerve. Baroreceptor discharges were identified by: (1) occlusion of the common carotid artery, which caused a decrease or abolition of discharges appearing synchronously with pulsatile pressure of the artery, and (2) making intravenous injections of noradrenaline, which increased the discharge frequency.

Intra-arterial injections of 5-HT (0.5-2 μg) induced an abrupt increase in baroreceptor discharges, as illustrated in Figure 10, in which two single units obtained from different animals were activated by the agent. A burst of discharges occurred about 0.5 s after the injection of 5-HT (2 μg). This excitatory effect on baroreceptor activity lasted for 3 s and thereafter, the spontaneously occurring discharges disappeared. The discharge re-appeared within 20 seconds. The block of discharges which previously occurred synchronously with pulsatile pressure of arterial blood pressure may have been due to a fall in blood pressure caused by 5-HT. However, 20 s after noradrenaline (2 μg/kg, i.v.), when the arterial pressure level was above the control, 5-HT also blocked baroreceptor discharges after inducing an intense increase in discharge frequency. Therefore, the excitatory and inhibitory phase of the response to 5-HT is most probably due to the direct action of 5-HT on the baroreceptor nerve ending.

These observations reveal that the response pattern of chemoreceptor activity to 5-HT is very similar to that of sinus baroreceptors.

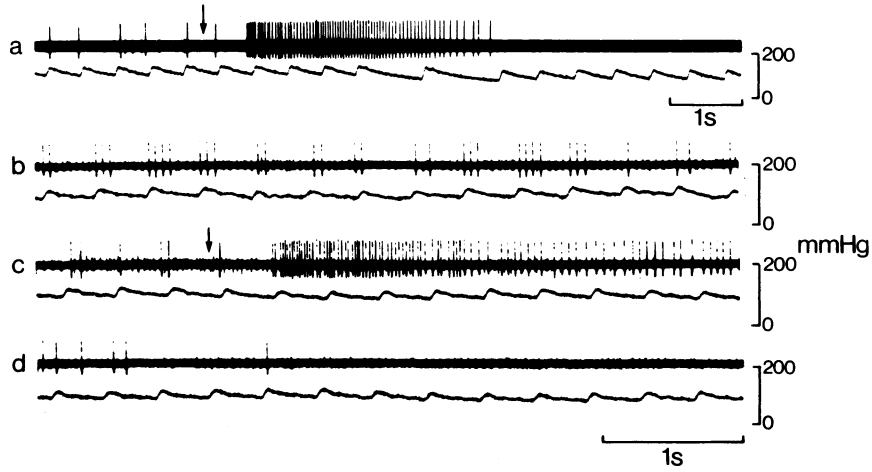

Figure 10 Effect of 5-hydroxytryptamine (5-HT) on baroreceptor activity; (a), (b), (c) and (d) show unitary responses of baroreceptor fibres obtained from different animals to 5-HT (2 μg in 0.25 ml Locke solution, i.a.) injected at arrows. (b), (c) and (d) are continuous records. Upper trace in each record shows sinus-baroreceptor fibre discharges. Lower trace, arterial blood pressure.

Discussion

The present experiments have demonstrated that intra-arterial injections of 5-HT induce a sharp and brief increase in chemoreceptor discharges followed by depression or block of naturally occurring discharges. Several experimental observations tend to indicate that the effects of 5-HT on chemoreceptor activity is most probably due to direct actions of 5-HT on the nerve endings of chemoreceptor afferent fibres: (1) relatively high doses of atropine and hexamethonium did not alter the response to 5-HT, indicating that a cholinergic mechanism is not involved in eliciting chemoreceptor activation produced by 5-HT; (2) a burst of chemoreceptor discharges occurred within 0.5 s after the start of the injection of 5-HT. This is characteristic of substances that act directly on sensory nerve endings, while substances that act on 'presynaptic' element, presumably the Type I cell and ischaemia induced by occlusion of the common carotid artery caused a gradual increase in chemoreceptor discharges (Nishi & Eyzaguirre, 1971; Andrew *et al.*, 1972; Eyzaguirre & Nishi, 1974; Nishi, unpublished observation); (3) the latency of the chemoreceptor discharge induced by 5-HT was approximately the same as that of baroreceptor activation produced by the agent, which presumably acts directly on nerve endings. In fact, 5-HT strongly stimulates sensory nerve endings in the rabbit aortic nerves (Douglas & Ritchie, 1957a), in the cat saphenous nerve (Douglas & Ritchie, 1957b; Fjallbrant & Iggo, 1959, 1961), in the rat saphenous nerve (Van Gelder, 1962) and in the hepatic nerve (Andrews, 1968). These considerations are based on the author's conviction that the glomus Type I cells are receptor cells and that the calyx-type endings impinging on them are sensory nerve endings (De Castro, 1951; Eyzaguirre *et al.*, 1972; Hess & Zapata, 1972; Nishi & Stensaas, 1974). However, the possibility that 5-HT may act directly on chemoreceptor free nerve endings in the carotid body (Biscoe, 1971) cannot be ruled out.

With regard to the biphasic response of chemoreceptors to 5-HT, a possible mechanism of action can be offered. The exogenous 5-HT may induce nerve ending depolarization with a rapid onset and, hence, initiate the nerve impulses at the non-myelinated portion of the sensory fibre or at the first node. However, once depolarization reaches a certain level, a cathodal type of block, or desensitization may occur a few seconds after the injection of 5-HT, and depolarization may further develop for another several seconds. When depolarization gradually subsides, the discharges may re-appear. This situation may also occur in the response to 5-HT during asphyxia and hence, induce a further increase in chemoreceptor discharges followed by sudden block of the discharges. Chemoreceptor responses to ACh and NaCN were slightly depressed when these substances were applied within a short period after 5-HT. The mechanism of the depressant action on chemoreceptor activation induced by ACh and NaCN is rather difficult to interpret. However,

part of this effect was probably due to sustained depolarization of the nerve endings induced by the previous 5-HT, since in the carotid body superfused with high K^+-solutions which depolarize the receptor elements (nerve endings and/or, presumably, glomus cells), the responses evoked by either ACh or NaCN become smaller than those in the control (Eyzaguirre & Nishi, unpublished observation). A somewhat similar situation can be seen in the cat superior cervical ganglion or other ganglia; 5-HT induces a depolarization with a rapid onset lasting a few seconds and the depolarization produced by small doses is accompanied by asynchronous discharges in the postganglionic nerve (Eccles & Libet, 1961; Herzler, 1961; Gyermek & Bindler, 1961; Jaramillo & Volle, 1968; Watson, 1970), while depression of ganglionic transmission occurs during the sustained depolarization elicited by high doses of 5-HT (Machová & Bŏska, 1969).

In the present experiments, repeated injections of 5-HT at short intervals, or a small dose after a large one depressed the responses to 5-HT. The loss of the excitatory effect of 5-HT is probably due to desensitization or tachyphylaxis which is a general characteristic of the action of 5-HT at many peripheral sites, e.g. in the guinea-pig taenia coli preparation (Bülbring & Burnstock, 1960; Born, 1962), or molluscan heart (Welsh, 1957). The present results are also consistent with earlier observations *in vivo* that the chemoreceptor reflex-response to the second dose of 5-HT applied within a period of about 5 min was greatly reduced or absent (Ginzel & Kottegoda, 1954).

With regard to a possible role of 5-HT present in the carotid body, the present experiments with reserpine have not been conclusive, since a quantitative estimate of the degree of depletion of 5-HT by the acute reserpine treatment was not made. However, large doses of reserpine (5-10 mg/kg), which caused a marked decrease in the content of catecholamines in the carotid body (Zapata *et al.*, 1969), may have also reduced the content of 5-HT as well, since in other tissues, acute reserpine-treatment produces considerable decreases in the content of catecholamines as well as 5-HT and furthermore, the time course of the decrease in the 5-HT content is very similar to that for catecholamines (see Carlsson, 1966). Thus, the present results have provided further evidence that 5-HT as well as catecholamines are not main excitatory 'transmitter' substances.

If 5-HT is an excitatory transmitter substance, spontaneously occurring discharges as well as chemoreceptor activation induced by NaCN or by asphyxia should have been depressed or blocked after reserpine-treatment. However, 20 h after a large dose of reserpine, chemoreceptor discharges still occurred, and NaCN as well as asphyxia produced marked chemoreceptor activation (Zapata *et al.*, 1969). Nevertheless, it is possible that 5-HT may play a more subtle role in chemoreceptor mechanisms, since chemoreceptors in the carotid body became more susceptible to 5-HT after reserpine. This may have been due to the denervation sensitization as occurs in other adrenergic structures where reserpine potentiates the response to catecholamines (Bein, 1956; Burn & Rand, 1958; Axelrod, 1964). The endogenous 5-HT could have local modulating effects and either excite or inhibit some receptor components (nerve endings and/or glomus Type I cells), depending upon the amounts of 5-HT released in intimate relationship to appropriate receptors. Another possibility is that endogenous 5-HT could act as a locally circulating hormone influencing the vascular permeability within the carotid body. In fact, 5-HT induces endothelial openings, the endothelial cells becoming partially disconnected along the intercellular junctions (Majno & Palade, 1961; Majno, Schoefl & Palade, 1961). If this is the case in the carotid body capillary, chemoreceptor function would be influenced, since the carotid body consists of networks of capillaries and also the chemoreceptors are very sensitive to changes in local circulation (Joel & Neil, 1963).

The author expresses his sincere gratitude to Dr Eyzaguirre, Department of Physiology, College of Medicine, University of Utah, U.S.A., and to Dr F. Takenaka, Department of Pharmacology, Kumamoto University, Medical School, Japan, for reading the manuscript and commenting on it. This work was supported by a grant, No. 821012, from the Japanese Education Ministry.

Methysergide was kindly supplied by Sandoz Ltd.

References

ANDREW, M.S., BLACK, S., COMROE, J.H. Jr. & JACOBS, L. (1972). Species difference in carotid body response of cat and dog to dopamine and serotonin. *Amer. J. Physiol.*, **223**, 1097-1102.

ANDREWS, W.H.H. (1968). Afferent impulses in the hepatic nerve of perfused livers elicited by 5-hydroxytryptamine and other substances. *Br. J. Pharmac.*, **32**, 421-422P.

AXELROD, J. (1964). The uptake and release of catecholamines and the effect of drugs. In *Biogenic Amines, Progress in Brain Research, Vol. 8,* ed. Himwich, H.E. & Himwich, W.A., pp. 81-89. Amsterdam: Springer.

BEIN, H.J. (1956). The pharmacology of Rauwolfia. *Pharmac. Rev.*, **8**, 435-483.

BISCOE, T.J. (1965). Some effects of drugs on the isolated superfused carotid body. *Nature, Lond.*, **208**, 294-295.

BISCOE, T.J. (1971). Carotid body: structure and function. *Physiol. Rev.*, **51**, 437-495.

BLACK, A.M.S. & TORRANCE, R.W. (1971). Respiratory oscillations in chemoreceptor discharge in the control of breathing. *Respirat. Physiol.*, **13**, 21-237.

BORN, G.V.R. (1962). The fate of 5-hydroxytryptamine in a smooth muscle and in connective tissue. *J. Physiol.*, **161**, 160-174.

BRADLEY, P.B. & WOLSTENCROFT, J.H. (1965). Actions of drugs on single neurons in the brain stem. *Br. med. Bull.*, **21**, 15-18.

BÜLBRING, E. & BURNSTOCK, G. (1960). Membrane potential changes associated with tachyphylaxis and potentiation of the response to stimulating drugs in smooth muscle. *Br. J. Pharmac. Chemother.*, **15**, 611-624.

BURN, J.H. & RAND, M.J. (1958). The action of sympathomimetic amines in animals treated with reserpine. *J. Physiol.*, **144**, 314-336.

CARLSSON, A. (1966). Drugs which block the storage of 5-hydroxytryptamine and related amines. In *5-Hydroxytryptamine and Related Indolealkylamines. Handbook of Experimental Pharmacology. Vol. 19,* ed. Eichler, O. & Farah, A., pp. 529-59. Berlin, Heiderberg, New York: Springer.

CHIOCCHIO, S.R., BISCARDI, A.M. & TRAMEZZANI, J.H. (1966). Catecholamines in the carotid body of the cat. *Nature, Lond.*, **212**, 834-835.

CHIOCCHIO, S.R., BISCARDI, A.M. & TRAMEZZANI, J.H. (1967). 5-Hydroxytryptamine in the carotid body of the cat. *Science,* **158**, 790-791.

CHIOCCHIO S.R., KING, M.P., CARBALLO, L. & ANGELAKOS, E.T. (1971). Monoamines in the carotid body cells of the cat. *J. Histochem. Cytochem.*, **19**, 621-626.

COMROE, J.H. Jr. & MORTIMER, L. (1964). The respiratory and cardiovascular responses of temporally separated aortic and carotid bodies to cyanide, nicotine, phenyldiguanide and serotonin. *J. Pharmac. exp. Ther.*, **146**, 33-41.

COMROE, J.H. Jr. (1966). *Physiology of Respiration.* Chicago: Year Book Medical Publishers Inc.

DE CASTRO, F. (1951). Sur la structure de la synapse dans les chemorecepteurs: leur mécanism d'excitation et röle dans la circulation sanguine locale. *Acta physiol. scand.*, **22**, 14-43.

DOUGLAS, W.W. & TOH, C.C. (1952). The effect of serotonin (5-hydroxytryptamine) on respiration in the dog. *J. Physiol.*, **117**, 71-72P.

DOUGLAS, W.W. & RITCHIE, J.M. (1957a). On excitation of non-medullated afferent fibres in the vagus and aortic nerves by pharmacological agents. *J. Physiol.*, **138**, 31-43.

DOUGLAS, W.W. & RITCHIE, J.M. (1957b). Discharges in non-medullated afferent fibres in the cat's saphenous nerve in response to touch and drugs. *J. Physiol.*, **139**, 9-10P.

ECCLES, R.M. & LIBET, B. (1961). Origin and blockade of the synaptic responses of curarized sympathetic ganglia. *J. Physiol.*, **157**, 484-503.

EYZAGUIRRE, C. & KOYANO, H. (1965). Effects of some pharmacological agents on chemoreceptor discharges. *J. Physiol.*, **178**, 410-438.

EYZAGUIRRE, C., NISHI, K. & FIDONE, S. (1972). Chemoreceptor synapses in the carotid body. *Fedn. Proc.*, **31**, 1385-1393.

EYZAGUIRRE, C. & NISHI, K. (1974). Further study on mass receptor potential of carotid body chemoreceptors. *J. Neurophysiol.*, **37**, 156-169.

EYZAGUIRRE, C. & ZAPATA, P. (1968). A discussion of possible transmitter or generator substances in the carotid body chemoreceptors. In *Arterial Chemoreceptors,* ed. Torrance, R.W., pp. 213-251. Oxford: Blackwell.

FILLENZ, M. & WOODS, R.I. (1966). Some observations on the rabbit carotid body. *J. Physiol.*, **186**, 39-40P.

FJALLBRANT, N. & IGGO, A. (1959). Cutaneous afferent fibres and pain-producing chemicals. *J. Physiol.*, **145**, 28-29P.

FJALLBRANT, N. & IGGO, A. (1961). The effect of histamine, 5-hydroxytryptamine and acetylcholine on cutaneous afferent fibres. *J. Physiol.*, **156**, 578-590.

GINZEL, K.H. & KOTTEGODA, S.R. (1954). The action of 5-hydroxytryptamine on arotic and carotid sinus receptors in the cat. *J. Physiol.*, **123**, 77-288.

GYERMEK, L. (1966). Drugs which antagonize 5-hydroxytryptamine and related indolealkylamines. In *5-Hydroxytryptamine and Related Indolealkylamines. Handbook of Experimental Pharmacology. Vol. 19*, ed. Eichler, O. & Farah, A., pp. 471-528. Berlin, Heiderberg, New York: Springer.

GYERMEK, L. & BINDLER, E. (1961). Blockade of the ganglionic stimulant action of 5-hydroxytryptamine. *J. Pharmac. exp. Ther.*, **135**, 344-348.

HAEFELY, W. (1972). Electrophysiology of the Adrenergic Neuron. In *Catecholamines,* ed. Blaschko, H. & Muscholl, E., pp. 661-725. Berlin, Heiderberg, New York: Springer.

HAMBERGER, B., RITZEN, M. & WERSALL, J. (1966). Demonstration of catecholamines and 5-hydroxytryptamine in the human carotid body. *J. Pharmac. exp. Ther.*, **152**, 197-201.

HERZLER, E.C. (1961). 5-Hydroxytryptamine and transmission in sympathetic ganglia. *Br. J. Pharmac. Chemother.*, **17**, 406-413.

HESS, A. & ZAPATA, P. (1972). Innervation of the cat carotid body: normal and experimental studies. *Fedn. Proc.*, **31**, 1365-1382.

HEYMANS, C. & NEIL, E. (1958). *Reflexogenic Areas of the Cardio-Vascular System,* London: Churchill.

I-LI CHEN, YATES, R.D. & DUNCAN, D. (1967). Electron microscope localization of biogenic amines in the carotid body. *J. Cell Biol.*, **35**, 40.

JACOBS, L. & COMROE, J.H. Jr. (1971). Reflex apnea, bradycardia, and hyptotension produced by serotonin and phenyldiguanide acting on the nodose ganglia of the cat. *Circulation Res.*, **29**, 145-155.

JARAMILLO, J. & VOLLE, R.L. (1968). A comparison of the ganglionic stimulating and blocking properties of some nicotinic drugs. *Arch. int. Pharmacodyn.*, **174**, 88-97.

JOELS, N. & NEIL, E. (1963). The excitation mechanism of the carotid body. *Br. med. Bull.*, **19**, 1-24.

KOBAYASHI, S. (1971). Comparative cytological studies of the carotid body. 1. Demonstration of monoamine-storing cells by correlated chromaffin reaction and fluorescence histochemistry. *Arch. Histol., Okayama,* **33**, 319-339.

KRNJEVIĆ, K. (1965). Actions of drugs on single neurons in the cerebral cortex. *Br. med. Bull.*, **21**, 10-14.

LEVER, J.D., LEWIS, P.R. & BOYD, J.D. (1959). Observations on the fine structure and histochemistry of the carotid body in the cat and rabbit. *J. Anat., Lond.*, **93**, 478-490.

MACHOVÁ, J. & BŎSKA, D. (1969). The effect of 5-hydroxytryptamine, diethylphenylpiperazium and acetylcholine on transmission and surface potential in the cat sympathetic ganglion. *Europ. J. Pharmac.*, 7, 152-158.

MAJNO, G. & PALADE, G.E. (1961). Studies on inflammation. The effect of histamine and serotonin on vascular permeability: an electron microscopy study. *J. biophys. biochem. Cytol.*, **11**, 571-605.

MAJNO, G., PALADE, G.E. & SCHOEFL, G.I. (1961). Effect of histamine and serotonin on vascular permeability. *Fedn. Proc.*, **20**, 119.

McCUBBIN, J.W., GREEN, J.H., SALMOIRAGHI, G.C. & PAGE, I.H. (1956). The chemoreceptor stimulant action of serotonin in dogs. *J. Pharmac. exp. Ther.*, **116**, 191-197.

NIEMI, M. & OJALA, K. (1964). Cytochemical demonstration of catecholamines in the human carotid body. *Nature, Lond.*, **203**, 539-540.

NISHI, K. & EYZAGUIRRE, C. (1971). The action of some cholinergic blockers on carotid body chemoreceptors *in vivo*. *Brain Res.*, **33**, 37-56.

NISHI, K. & STENSAAS, L.J. (1974). The ultrastructure and source of nerve endings in the carotid body. *Cell Tiss. Res.*, **154**, 303-320.

SAMPSON, S.R. (1972). Mechanism of efferent inhibition of carotid body chemoreceptors in the cat. *Brain Res.*, **45**, 266-270.

THOENEN, H., HAEFELY, W. & GEY, K.F. (1967). Quantitative aspects of the replacement of norepinephrine by dopamine as a sympathetic transmission after inhibition of dopamine-β-hydroxylase by disulfiram. *J. Pharmac. exp. Ther.*, **156**, 46-251.

TRENDELENBURG, U. (1958). The 5-hydroxytryptamine receptors of the cat superior cervical ganglion. In *5-Hydroxytryptamine*, ed. Lewis, G.P., pp. 136-139. London: Pergamon Press.

TRENDELENBURG, U. (1959). Non-nicotinic ganglion-stimulating substances. *Fedn. Proc.*, **18**, 1001-1005.

VAN GELDER, N.M. (1962). Effects on sensory nerves of injecting 5-hydroxytryptamine into the skin. *Nature, Lond.*, **195**, 185-186.

WATSON, P.J. (1970). Drug receptor sites in the isolated superior cervical ganglion of the rat. *Europ. J. Pharmac.*, **12**, 183-193.

WELSH, J.H. (1957). Serotonin as a possible neurohumoral agent: evidence obtained in lower animals. *Ann. N.Y. Acad. Sci.*, **66**, 618-630.

ZAPATA, P., HESS, A., BLISS, E.L. & EYZAGUIRRE, C. (1969). Chemical, electron microscopic and physiological observations on the role of catecholamines in the carotid body. *Brain Res.*, **14**, 473-495.

(Received February 10, 1975.
Revised March 30, 1975.)