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Effects of the antagonists MDL 72222 and ketanserin on responses of cat carotid body chemoreceptors to 5-hydroxytryptamine

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1 The effects of intracarotid (i.c.) injections of 5-hydroxytryptamine (5-HT; 1–50 μg) on carotid chemoreceptor activity recorded from the carotid sinus nerve have been studied in anaesthetized cats.

2 Three separate components in the complex response of the chemoreceptors to injected 5-HT were identified. Firstly, a transient burst of activity was obtained during the injection period in 56% of the recordings. Secondly, in all the recordings a period of chemodepression commenced a few seconds after completing the injection and was usually dose-related. Thirdly, a delayed longer-lasting chemoexcitation occurred in many experiments, concomitant with a fall in systemic blood pressure.

3 The neuronal 5-HT receptor antagonist MDL 72222 (10–100 μg kg^{-1}, i.c.) virtually abolished the transient chemoexcitation evoked during 5-HT injections and also significantly increased the mean ID_{50} for 5-HT-induced chemodepression; in 37% of recordings 5-HT caused a dose-related chemoexcitation after the high dose of MDL 72222. Neither the delayed chemoexcitation nor the hypotension caused by 5-HT were much affected by the antagonist. MDL 72222 itself had a biphasic effect on chemosensory discharge, causing depression followed by a delayed excitation.

4 The 5-HT_2-receptor antagonist ketanserin (100 μg kg^{-1}, i.c.) had no appreciable effect on the transient chemoexcitation evoked during 5-HT injections and caused a slight but significant increase in the mean ID_{50} for 5-HT-induced chemodepression. The delayed chemoexcitation and accompanying hypotension associated with 5-HT were both substantially reduced or abolished by the antagonist. Ketanserin itself caused a short-lasting period of chemoexcitation.

5 All the effects of injected 5-HT on chemosensory discharge could be abolished by the combination of MDL 72222 and ketanserin (100 μg kg^{-1}, i.c.).

6 Neither MDL 72222 nor ketanserin had any significant effect upon the response of the carotid chemoreceptors to hypoxia. The rate at which discharge increased, and also the steady-state discharge before and during hypoxia, were unaffected by the antagonists, alone or in combination.

7 At least two types of 5-HT receptor appeared to be involved in the response of carotid body chemoreceptors to 5-HT. Transient excitation and chemodepression were mediated via MDL 72222-sensitive (peripheral neuronal) receptors whereas the delayed chemoexcitation and associated hypotension involved a ketanserin-sensitive, presumably 5-HT_2-, receptor. It appears unlikely that 5-HT plays a crucial role in chemoreception.

Introduction

Although it is well known that 5-hydroxytryptamine (5-HT, serotonin) is present in the carotid body of many species, including the cat (e.g. Chiocchio *et al.*, 1967), its physiological role there remains to be determined. The effects of exogenous 5-HT on chemosensory activity recorded from the carotid sinus nerve have been studied in anaesthetized cats (Black *et al.*, 1972; Nishi, 1975; Docherty & McQueen, 1978). A complex response is obtained following the intracarotid injection of 5-HT, with a common pattern being a brief period of chemoexcitation followed by a longer-lasting depression of background chemoreceptor discharge. Nishi (1975) found that the response to 5-HT was unaffected by

atropine or hexamethonium, which eliminates involvement of acetylcholine (ACh) receptors, but the putative 5-HT antagonists lysergic acid diethylamide (LSD), gramine and methysergide were also without effect on the response of the carotid chemoreceptors to 5-HT. Categorization of 5-HT receptors in the peripheral nervous system is a complex problem (see Gyermek, 1961; Wallis, 1981) and the value of studies with drugs such as methysergide or LSD in helping to characterize the carotid body 5-HT receptors has been questionable because they lack specificity as 5-HT antagonists. However, the recent advent of more specific 5-HT antagonists such as MDL 72222, shown to be potent in antagonizing the actions of 5-HT at peripheral neuronal sites (e.g. fibres mediating the Bezold-Jarisch reflex in rat – Fozard, 1984), and ketanserin, which is reputed to be a highly selective antagonist at 5-HT_2-receptor sites but inactive at 5-HT_1-receptor sites (Leysen *et al.*, 1981), prompted us to study their effects on the cat carotid chemoreceptors. The aim was to attempt a characterization of 5-HT receptors involved in the response of cat carotid chemoreceptors to 5-HT and, by investigating the influence of the antagonists on responses of the chemoreceptors to a physiological stimulus, hypoxia, obtain information regarding the physiological role of 5-HT in the carotid body.

A preliminary account of some of the results has previously been presented (Kirby & McQueen, 1984).

Methods

Experiments were performed on fifteen cats of either sex, weighing between 2.3 and 3.9 kg, median weight 3.0 kg. Animals were anaesthetized with α-chloralose (65–70 mg kg^{-1}, intravenously) following induction with halothane (5% in oxygen) and supplements of chloralose were administered intravenously as required. In two experiments pentobarbitone (42 mg kg^{-1}, intraperitoneally) was used instead of α-chloralose.

Full details of the experimental procedures have been given previously (McQueen, 1977; Docherty & McQueen, 1978) so only a brief description is provided here. The carotid sinus region on one side was dissected and the ganglioglomerular nerves, which carry the sympathetic nerve supply from the superior cervical ganglion to the carotid body, were cut. The cats were artificially ventilated with room air, apart from periods when hypoxic gas mixtures were used. Gallamine triethiodide (3 mg kg^{-1}) was administered intravenously to prevent spontaneous and drug-induced muscle movements. Drugs were dissolved in modified Locke solution or in 0.9% w/v NaCl solution (saline) and injected, in volumes of 0.1 ml, into the common carotid artery ipsilateral to the sinus nerve from which electrical activity was recorded. They were washed in with 0.2 ml Locke solution which had been bubbled with 5% CO_2:95% air at 37°C; injections were generally completed within 1–2 s.

Electrical activity of single or multiple (2–4) chemoreceptor units was recorded from the peripheral cut end of the sinus nerve and stored on tape for subsequent analysis using a pulse height voltage discriminator linked to a microcomputer (McQueen *et al.*, 1984). The units were confirmed as chemoreceptors by their random pattern of discharge, their increase in discharge frequency following injection of sodium cyanide (2.5 μg) into the ipsilateral common carotid artery or in response to hypoxia (breathing 10% oxygen in nitrogen), and by the depression of discharge in response to hyperoxia (breathing 100% oxygen).

Data analysis

A plot of chemosensory discharge (counts per 0.1 s bin) against time was made for each test, and the change in discharge frequency ($\bar{x}$ c.p.s.) from the pre-injection control frequency calculated. In order to standardize results from experiments with different absolute discharge frequencies, the response occurring in the first 5 s of the post-injection period was expressed as a percentage change from control level and plotted against $\log_{10}$ dose. From lines fitted to the dose-response data it was possible to calculate the dose causing a particular response (e.g. ID_{50}, dose causing a 50% reduction in control discharge) and obtain a mean value by pooling data from different experiments.

Hypoxia

The animals were made hypoxic by switching the inspired gas from air to 10% O_2 plus 90% N_2 for 4 min. For each test the control (air-breathing) discharge and steady-state discharge (hypoxia) were measured and arterial blood samples taken before and 3.5 min after onset of the hypoxic stimulus for blood gas analysis. Discharge was measured over consecutive 15 s periods and plotted against time, and a straight line fitted to the values obtained when discharge was increasing in response to the hypoxic stimulus (i.e. until a steady-state maximum (100%) or plateau discharge was obtained). The slope of this line provided an index of the rate of increase in chemoreceptor discharge in response to hypoxia, and was expressed as % max s^{-1}.

Statistics

Mean values are given ± s.e.mean. Statistical analysis of differences between means was carried out using the Wilcoxon two-sample test and the null hypothesis rejected at the 0.05 level of probability (2-tailed).

Drugs

The following compounds were used, and doses are expressed in terms of the salt: 5-hydroxytryptamine creatinine sulphate complex, dopamine hydrochloride (Sigma); MDL 72222 (1αH, 3α, 5αH-tropan-3-yl 3,5-dichlorobenzoate) methanesulphonate salt, kindly donated by Merrell International, Strasbourg; domperidone and ketanserin, tartaric acid salt, kindly donated by Janssen Pharmaceuticals, Beerse, Belgium.

Results

5-HT injections

Sixteen recordings of chemosensory activity were obtained, and intracarotid injection of 5-HT (1–50 μg) consistently caused a *depression* of chemosensory discharge which lasted for 3–15 s. The effect was dose-related in twelve of the recordings (75%), as shown in Figure 1. In the other four experiments chemodepression was not clearly related to dose. 5-HT doses of less than 1 μg had only slight effects on background discharge and these did not differ significantly from those associated with injection of the drug vehicle; the latter had variable effects on chemosensory discharge causing, on average, a 20.5% reduction during the 5 s post-injection period. In nine recordings (56%) chemodepression

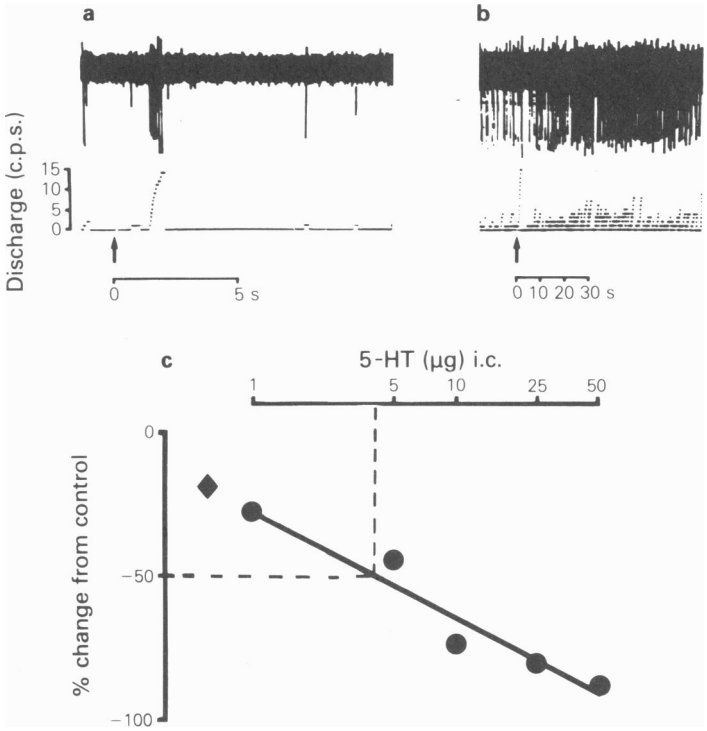

Figure 1 (a and b) Neurograms showing the effects of a single intracarotid (i.c.) injection of 5-hydroxytryptamine (5-HT; 10 μg, at arrow) on chemosensory discharge (3 units) and illustrating in (a) the initial burst of activity which is followed by a period of relative inhibition. A delayed phase of excitation can be seen in (b), which shows the same test displayed at a slower sweep speed. A ramped output below the neurograms gives the number of action potentials counted cumulatively in successive 1 s intervals. (c) Dose-response data showing the relationship between 5-HT dose and the percentage change in chemosensory discharge (from pre-injection control levels) that occurred during the first 5 s after injecting 5-HT. The straight line was fitted to the data by the least squares method, and the ID_{50} (i.e. dose of 5-HT causing a 50% depression of discharge – see broken lines) determined. The mean of the five individual control discharge values, from which the percentage changes were calculated, was 8.03 ± 0.26 c.p.s.. (◆) Effect associated with injection of the drug vehicle.

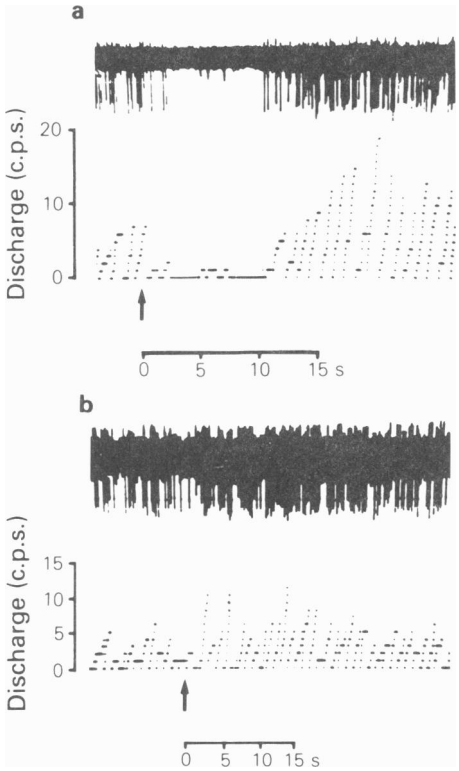

Figure 2 Neurograms of chemosensory discharge illustrating the response to injecting, at the arrow, (a) MDL 72222 (100 $\mu g\,kg^{-1}$, i.c.) and, from a separate experiment, (b) ketanserin (100 $\mu g\,kg^{-1}$, i.c.). The ramped trace below each neurogram gives the number of action potentials counted cumulatively in 1 s intervals.

was preceded by a transient burst of chemoreceptor action potentials, usually occurring within the injection period (Figure 1). The threshold for this more variable *excitatory* effect ($\approx 10\,\mu g$) was generally higher than that for chemodepression, but although discharge increased substantially, by 800–1000% in some experiments, a clear dose-response relationship was obtained in only three of the experiments, and the response appeared to be subject to tachyphylaxis. In the remaining 25% of recordings in which 5-HT did not cause transient excitation, chemodepression was still obtained. The averaged ID_{50} for the dose-dependent chemodepression in the recordings where the effect was dose-related was $5.8 \pm 1.9\,\mu g$ ($n = 12$).

In many of the experiments a *delayed increase* in chemosensory discharge was observed following the chemodepression (Figure 1). This effect was rather variable, lasted for 10–60 s, and had no consistent dose-response relationship. It appeared to be associated with the fall in systemic blood pressure that occurred following 5-HT injection (Figure 5).

Effects of the antagonist MDL 72222

Intracarotid injection of MDL 72222 (10–100 $\mu g\,kg^{-1}$) caused a depression of background chemosensory discharge which was followed by a delayed increase in discharge frequency (Figure 2a). No chemoexcitation was observed during the period of injection, and systemic blood pressure was not significantly affected by the antagonist. MDL 72222 (10 $\mu g\,kg^{-1}$, i.c.) was studied on seven recordings and in each case the dose-response line relating dose of

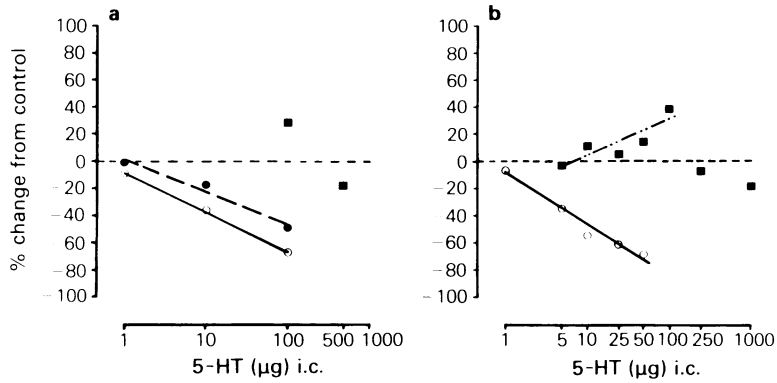

Figure 3 (a) Chemodepressant effect of 5-hydroxytryptamine (5-HT) injected before (○——○) and after (●----●) a low dose of MDL 72222 (10 $\mu g\,kg^{-1}$, i.c.). The rightward shift in the $\log_{10}$ dose-response curve caused by the antagonist is shown. An additional dose of MDL 72222 (100 $\mu g\,kg^{-1}$, i.c.) caused a further shift upwards and to the right (■). (b) In a separate experiment the higher dose of MDL 72222 (100 $\mu g\,kg^{-1}$, i.c.) completely abolished the 5-HT-induced chemodepression (■···■) and slight chemoexcitation was obtained in response to the lower doses of 5-HT. Lines were fitted to the data by the method of least squares.

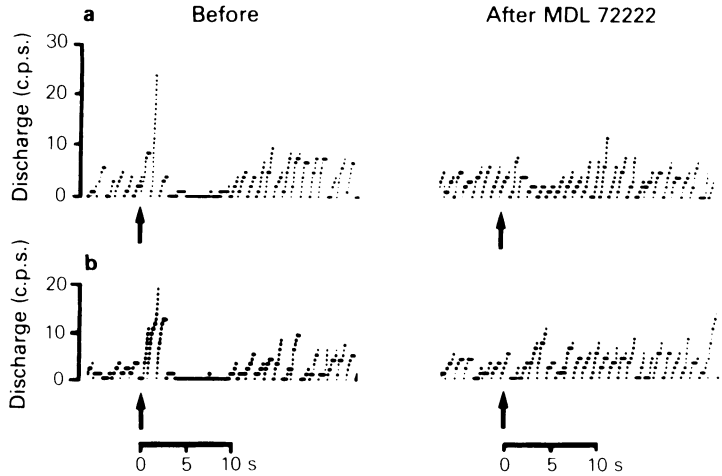

Figure 4 Responses of chemoreceptors (multi-unit recordings) to intracarotid injections of 5-hydroxytryptamine ((a) 5 and (b) 25 μg at arrows) before and after MDL 72222 (100 μg kg^{-1}, i.c.). The initial transient chemoexcitation and the subsequent depression of chemoreceptor discharge were both virtually abolished by the antagonist, and chemoexcitation became evident within the 5 s post-injection period. The ramps show the number of action potentials counted cumulatively in 1 s intervals.

5-HT to chemodepression was shifted upwards and to the right (Figure 3), and the mean ID_{50} was increased to 49.4 ± 33.6 μg ($n = 7$; $P < 0.05$ with respect to controls). When a dose of 100 μg kg^{-1} was given (8 recordings, in 6 of which it followed the lower dose) the ID_{50} increased to 638 ± 408 μg in five (63%) of the recordings. In the other three cases (37%) a dose-dependent chemoexcitation was obtained in the 5 s post-injection period (Figure 3), and no chemodepression occurred unless very high doses of 5-HT (250–1000 μg) were injected. It was possible to calculate an ED_{30} (dose causing 30% increase in discharge above pre-injection level), and this was 103.1 ± 9.0 μg ($n = 3$).

During the nine experiments in which 5-HT caused an initial transient excitation, this part of the response was substantially reduced (totally abolished in two recordings – 22%), by the lower dose of

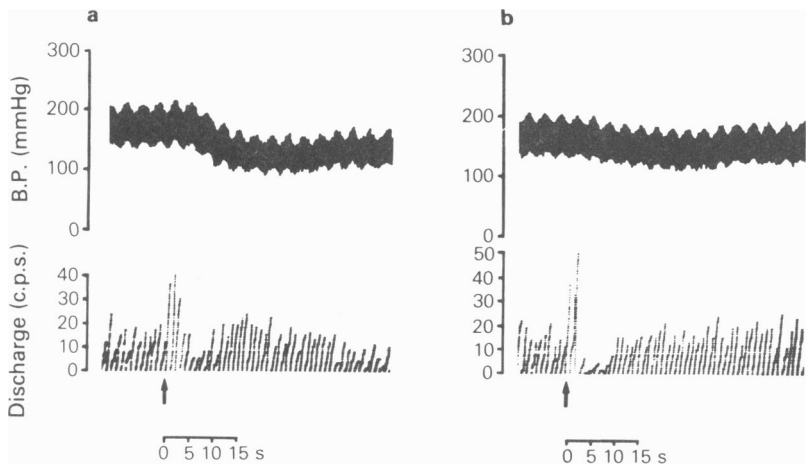

Figure 5 (a) Injection of 5-hydroxytryptamine (5-HT) injected (10 μg i.c., at arrow) affected chemosensory discharge (lower trace, count of action potentials per 1 s interval) and also caused a fall in systemic blood pressure (upper trace). (b) After administering ketanserin (100 μg kg^{-1}, i.c.) the same dose of 5-HT had much less effect upon blood pressure and there was less delayed chemoexcitation 10–15 s after the injection. However, both the initial burst of activity and the subsequent chemodepression were relatively unaffected by the antagonist.

MDL 72222 ($10\,\mu g\,kg^{-1}$), and further reduced or abolished by the higher dose ($100\,\mu g\,kg^{-1}$) – see Figure 4. In one experiment the 5-HT-induced transient chemoexcitation appeared only after MDL 72222 ($10\,\mu g\,kg^{-1}$) and was abolished by adding the higher dose ($100\,\mu g\,kg^{-1}$) of antagonist. The delayed or secondary chemoexcitation, which was more obvious following higher doses of 5-HT, generally increased in magnitude and became more rapid in onset after MDL 72222 ($10-100\,\mu g\,kg^{-1}$), as shown in Figure 6a.

Effects of the antagonist ketanserin

Intracarotid injection of ketanserin ($100\,\mu g\,kg^{-1}$) increased chemosensory discharge in the five recordings studied, an effect which lasted for 10–30 s (Figure 2b), and caused a longer-lasting fall in systemic blood pressure. There was no depression of discharge following ketanserin and no transient chemoexcitation during the injection period. The initial excitation caused by 5-HT was present in three of these recordings and was unaffected by the antagonist (Figure 5). The ID_{50} for 5-HT-induced chemodepression was $14.2 \pm 5.0\,\mu g$ ($n = 4$), which represents a small but significant ($P < 0.05$) decrease in the average response after ketanserin – although much less marked than the antagonism caused by MDL 72222 ($10\,\mu g\,kg^{-1}$), and ketanserin had little or no influence in some of the tests (e.g. Figure 5). In all five recordings the delayed increase in discharge was substantially reduced, as was the hypotensive effect of 5-HT (Figure 5).

Effects of the two antagonists in combination

In five experiments where MDL 72222 ($100\,\mu g\,kg^{-1}$) was administered after ketanserin ($100\,\mu g\,kg^{-1}$) the normal responses to 5-HT injections were absent. Dose-response data for the first 5 s of the responses gave lines of such shallow slope that meaningful ID_{50} or ED_{30} values could only be obtained by extrapolation far beyond the range of doses that could feasibly be used in the experiments, and these were not considered to be meaningful. This was also the case in the five experiments where ketanserin ($100\,\mu g\,kg^{-1}$) was injected after MDL 72222 ($100\,\mu g\,kg^{-1}$).

Responses to dopamine, and the effects of domperidone

The chemodepressant effect of dopamine (0.1–$10\,\mu g$, i.c.) was obtained in all 16 recordings and was unaffected by either ketanserin or MDL 72222 (Figure 6). The dopamine D_2-receptor antagonist domperidone ($10-100\,\mu g\,kg$, i.c.$^{-1}$) was injected in

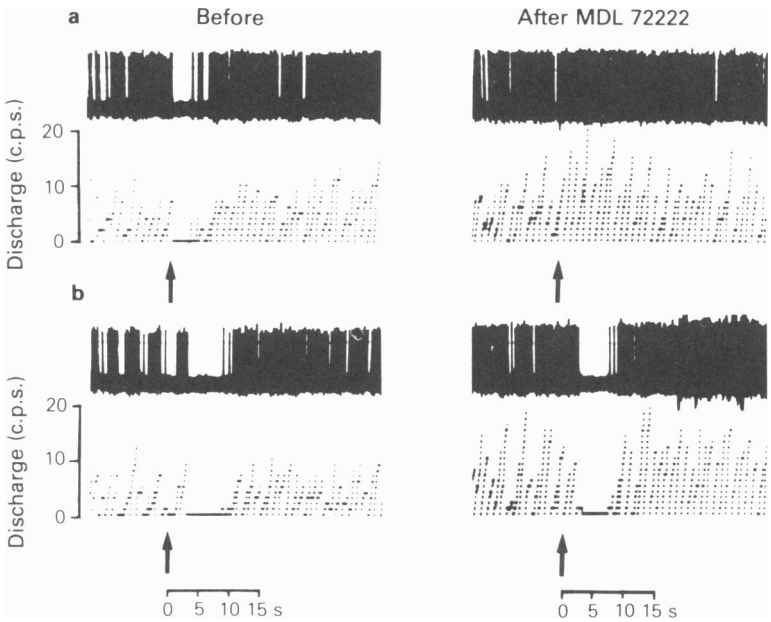

Figure 6 Neurograms showing the response of a single chemoreceptor unit to injections (arrows) of (a) 5-hydroxytryptamine $25\,\mu g$, i.c.) and (b) dopamine ($1\,\mu g$, i.c.) before and after administering MDL 72222 ($100\,\mu g\,kg^{-1}$, i.c.). It can be seen that, whereas the chemodepression evoked by 5-HT was greatly reduced by the antagonist, the dopamine-induced effect was unaltered. The ramps show the number of action potentials counted cumulatively in 1 s intervals.

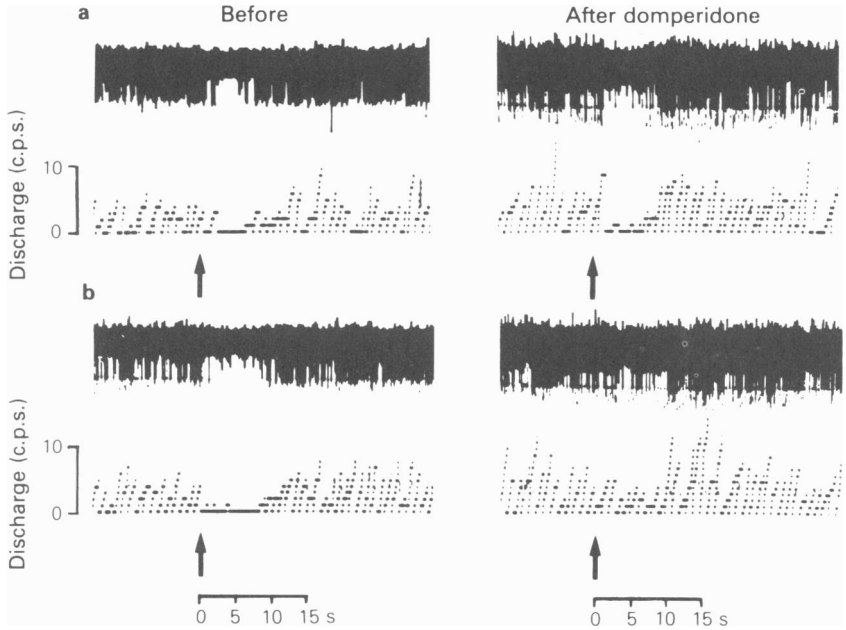

Figure 7 Neurograms showing the responses of chemoreceptor units to injections (arrows) of (a) 5-hydroxytryptamine 10 μg, i.c.) and (b) dopamine (1 μg, i.c.) before and after administering the dopamine antagonist domperidone (10 μg kg^{-1}, i.c.). The antagonist virtually abolished the chemodepressant effect of dopamine but had no appreciable effect upon the response evoked by 5-HT. The ramps show the number of action potentials counted cumulatively in 1 s intervals.

eight preparations and reduced the depressant effect of dopamine on the chemoreceptors without altering the responses to 5-HT (Figure 7), whether injected before (4 experiments) or after (4 experiments) the 5-HT antagonist(s). ID_{50} values for 5-HT-induced chemodepression obtained after domperidone alone were 10.8 ± 2.5 μg ($n = 4$) and 8.1 ± 3.1 μg ($n = 2$) for the 10 and 100 μg kg^{-1} doses, respectively ($P > 0.05$ in comparison with controls).

Responses to physiological (hypoxic) stimulation

The effect of hypoxic stimulation was studied in 14 experiments, and chemoreceptor responses obtained before and after administering MDL 72222 or ketanserin.

MDL 72222 Control chemosensory discharge frequency averaged 6.8 ± 1.5 c.p.s. on air, and increased to 26.8 ± 5.3 c.p.s. on 10% O_2 ($n = 6$). After MDL 72222 (10 μg kg^{-1}) background discharge on air increased to 10.8 ± 3.2 c.p.s., and rose to 27.3 ± 5.8 c.p.s. ($n = 6$) on 10% O_2. The slope of the line relating discharge to time during the period of increasing discharge was 1.08 ± 0.11% max s^{-1} before, and 1.04 ± 0.12 after MDL 72222. The time taken to reach the plateau or steady state discharge (i.e. maximum or 100%) was 112 ± 6 s before, and 109 ± 11 s after the low dose of MDL. A higher dose of MDL 72222 (100 μg kg^{-1}) was studied in 12 experiments (see Figure 8) in three of which ketanserin had previously been injected. Background discharge on air was 5.3 ± 1.0 c.p.s., and it increased to 21.9 ± 3.2 c.p.s. on 10% O_2, the slope being 1.02 ± 0.07% max s^{-1} and the time taken to reach plateau (max) 114 ± 4 s. After MDL 72222 background discharge was 7.7 ± 1.8 c.p.s., discharge on 10% O_2 26.3 ± 5.5, slope 1.08 ± 0.11, and the time taken to reach plateau 116 ± 7 s. None of these parameters was significantly different from control values, following either dose of MDL 72222.

Ketanserin In 6 hypoxia tests performed immediately before the injection of ketanserin (in one of which MDL 72222 had been administered previously) background activity in the pre-ketanserin control state was 6.9 ± 2.0 c.p.s., and increased to 25.6 ± 6.5 c.p.s. on 10% O_2, the slope of the line being 0.99 ± 0.06% max s^{-1}, reaching the plateau or maximum value 112 ± 6 s after changing gases. Following ketanserin (100 μg kg^{-1}) five hypoxia tests were carried out, and there was no significant change

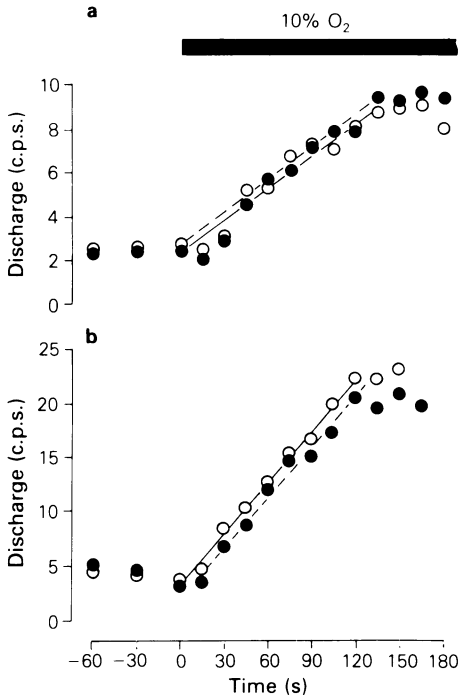

Figure 8 Increase in chemoreceptor discharge caused by ventilating the animals with a hypoxic gas mixture (10% O_2:90% N_2 during the 4 min period starting at 0 s, black bar) instead of room air. (a) Shows the discharge averaged over 15 s intervals, obtained before (○——○) and after (●---●) administering ketanserin (100 $\mu g\,kg^{-1}$, i.c.). (b) Shows the responses obtained, from a separate experiment, before (○——○) and after (●---●) administering MDL 72222 (100 $\mu g\,kg^{-1}$, i.c.). Lines were fitted to the data in the ranges shown by using the least squares method. Neither of the antagonists had any significant effect upon the response to hypoxia.

in any of the parameters (Figure 8). Background discharge was 6.9 ± 2.0 c.p.s., increasing to 26.0 ± 9.5 during 10% O_2, the slope was 1.02 ± 0.14% max s^{-1}, and time to plateau was 116 ± 17 s.

The mean values of arterial blood gas tensions and pH during air breathing (Pa_{O_2} 12.7 ± 0.7 kPa; Pa_{CO_2} 4.3 ± 0.1 kPa; pH 7.30 ± 0.01, $n = 17$) showed no significant differences between control values and those obtained after the administration of antagonists. Similarly, when the animals were made hypoxic, there were no significant differences in blood gas tensions and pH between the values obtained before (Pa_{O_2} 4.8 ± 0.2 kPa; Pa_{CO_2} 4.1 ± 0.2 kPa; pH 7.32 ± 0.01, $n = 17$) and after administration of the antagonists.

Discussion

The present results show that 5-HT has complex and somewhat variable effects upon chemosensory discharge in anaesthetized cats. We identified three separate components in the response to 5-HT and found that the 5-HT antagonists MDL 72222 and ketanserin selectively affected different parts of the response. Previous studies using respiration as an index have provided evidence for both inhibitory and excitatory effects of 5-HT on chemoreceptors in various species including cat (Page, 1952; Douglas & Toh, 1953; Ginzel & Kottegoda, 1954). 5-HT also induces complex neurogenic and circulatory effects which influence respiration independently of the chemoreceptors (Mott & Paintal, 1953; Comroe *et al.*, 1953), which makes the respiratory effect a poor index of chemoreceptor activity. We shall confine ourselves to a consideration of neural data, looking at the effects of antagonists on each of the components of the chemoreceptor response to 5-HT.

Chemoexcitation

Transient chemoexcitation occurred during the injection period in about half the recordings, and this effect has previously been described by Black *et al.* (1972), Nishi (1975), and Docherty & McQueen (1978). The increase was only occasionally dose-related and seemed subject to tachyphylaxis. The rapid onset suggests a direct or perhaps indirect (via release of endogenous substance(s)) action on the sensory nerve fibres (Eyzaguirre & Nishi, 1974), and makes it unlikely to be due to vascular effects of 5-HT. The possible influences on the chemoreceptors of increased sympathetic activity arising from the ganglion-stimulating action of 5-HT (Trendelenburg, 1958) were prevented by sectioning the ganglioglomerular nerves. This does not exclude the possibility that 5-HT may release noradrenaline from the terminals of sympathetic nerves within the carotid body; such an action might be expected to reduce blood flow through the carotid body and could cause a *delayed* increase in discharge. The finding that transient excitation was not obtained in all recordings, in accord with Black *et al.* (1972), could be because fibres differ in their sensitivity: unmyelinated fibres may be more sensitive than myelinated, or the concentration of 5-HT at the receptor site following intracarotid injection may vary between experiments. Tachyphylaxis to 5-HT cannot explain the absence of excitation in response to the initial doses of 5-HT, although in recordings where excitation was occurring, repeated administration of 5-HT in high doses did tend to attenuate the response and may explain why there was no dose-response relationship in many experiments. Nishi

(1975), who evidently found transient chemoexcitation by 5-HT to be more common and induced by lower doses than was our experience, reported that various antagonists were ineffective in blocking the response. He was unable to characterize the receptor responsible for excitation, although he did exclude direct or indirect involvement of nicotinic and muscarinic ACh receptors. Docherty & McQueen (1978) found that the dopamine antagonist α-flupenthixol could reduce excitatory responses to 5-HT, but inconsistently. We have not studied putative 5-HT antagonists such as LSD or methysergide because of concern over their specificity, but instead used the newer 5-HT antagonists MDL 72222 and ketanserin. The latter had no appreciable effect on the transient chemoexcitation but MDL 72222 inhibited it. Thus in some, but not all recordings of chemoreceptor activity, 5-HT increases discharge transiently and this occurs via actions, directly or indirectly mediated, at a receptor, presumably within the carotid body and perhaps associated with sensory nerve endings, which is sensitive to the antagonist MDL 72222. Recent studies have shown that MDL 72222 blocks the excitatory action of 5-HT on the cell bodies of rabbit vagal primary afferents (Azami *et al.*, 1984), and this evidence supports the concept of 5-HT acting on neuronal or sensory receptors in the carotid body. The fact that MDL 72222 itself had some 5-HT-like actions could mean the drug is a partial agonist.

Chemodepression

5-HT caused a short-lasting period of chemodepression which commenced almost immediately upon completion of the injection and was dose-related in the majority of experiments. Again, the rapid onset of the effect makes it unlikely to be secondary to vascular changes caused by 5-HT. It was the most commonly encountered component of the response and occurred regardless of whether or not the initial excitation was present. Depression of chemoreceptor discharge has been demonstrated previously (Black *et al.*, 1972; Nishi, 1975; Docherty & McQueen, 1978). Earlier studies showed that very high doses of α-flupenthixol reduced the relative inhibition caused by 5-HT (Docherty & McQueen, 1978), but none of the putative 5-HT antagonists examined by Nishi (1975) had any effect. His suggestion that depression might be secondary to the initial excitation seems unlikely, for if this were the case, chemodepression should not occur in the absence of an initial depolarization, yet it did in our experiments. We found that ketanserin had a rather variable effect upon 5-HT-induced chemodepression, usually causing a slight reduction (although sometimes a potentiation) of the effect. In contrast, MDL 72222 caused a substantial dose-related antagonism of chemodepression, as shown by the increase in ID_{50} values, and higher doses ($100\,\mu g\,kg^{-1}$) could completely abolish the response, unmasking an excitatory component. Dopamine also causes chemodepression when injected in cats (Docherty & McQueen, 1978), and in view of the fact that high doses of α-flupenthixol can reduce responses to 5-HT as well as to dopamine, we examined the responses to dopamine and 5-HT before and after administering the selective dopamine D_2-receptor (Kebabian & Calne, 1979) antagonist, domperidone. Domperidone had no significant effect on any phase of the chemoreceptor response to 5-HT when given in doses which substantially reduced dopamine-induced chemodepression, and responses to dopamine were unaffected by either MDL 72222 or ketanserin. Thus, we can conclude that chemodepression evoked by 5-HT does not involve a dopamine D_2-receptor and is mainly mediated by mechanisms which are sensitive to MDL 72222. The results with ketanserin could mean that a small part of the depression is attributable to actions on 5-HT_2-receptors, assuming the antagonist is selective and does not affect MDL 72222-sensitive sites in the doses studied. Whether the depression of discharge results from direct actions of 5-HT, or is secondary to the release of an inhibitory substance, cannot be determined from our study.

Delayed excitation

The final component of the response to injected 5-HT was a delayed (10–30 s) increase in discharge that lasted longer than any of the other components. However, it was very variable and tended to be concurrent with the fall in blood pressure caused by 5-HT. The antagonist MDL 72222 had no effect on blood pressure responses to 5-HT or on the delayed excitation whereas ketanserin inhibited both the hypotensive effect and the increase in discharge. The chemoexcitation caused by ketanserin alone may reflect some partial agonist activity of the drug. We cannot tell from the data whether the delayed chemoexcitation was due to the hypotension caused by 5-HT, or resulted from actions of the amine on 5-HT_2-receptors in the carotid body, perhaps associated with the vasculature (Leysen *et al.*, 1981) or the nerves (e.g. sympathetic terminals – see earlier discussion). Further experiments are needed to resolve the matter.

Classification of 5-HT receptors

In the peripheral nervous system classification of 5-HT receptors is complicated (see Wallis, 1981) and, in addition, new antagonists such as MDL 72222

have yet to be fully characterized *in vivo*. Accordingly, we can only conclude that at least two 5-HT receptors appear to be responsible for changes in carotid chemosensory discharge evoked by injected 5-HT. It is not possible to say whether or not the transient excitation and depression are mediated through a common MDL 72222-sensitive mechanism, but overall our findings are consistent with reports showing that MDL 72222 is a selective antagonist of responses mediated through 5-HT receptors on peripheral nerves (Fozard, 1984) and that ketanserin appears to be an effective antagonist at vascular 5-HT_2-receptors (Leysen *et al.*, 1981).

Physiological stimulation

Although the combination of MDL 72222 and ketanserin antagonized the effects of exogenous 5-HT on chemosensory discharge, the response of the chemoreceptors to physiological stimulation by hypoxia was unaltered. Assuming the antagonists studied reach effective concentrations at sites within the carotid body where locally-released 5-HT acts, the implication is that endogenous 5-HT has no vital role in the mechanism of chemoreception. However, the possibility that 5-HT might exert subtle influences, perhaps as a modulator or co-transmitter, that were not detected in these experiments, cannot entirely be excluded. Neuronal co-storage of 5-HT with polypeptides, some of which are present in the cat carotid body (e.g. substance P; Cuello & McQueen, 1980), and may be neurotransmitters (see Hökfelt *et al.*, 1980), could mean that if 5-HT is involved in chemoreception it may function more as a modulator than as a 'primary' transmitter. The conditions of our experiments do not allow us to reach any conclusions on this possibility, nor on the question of whether 5-HT may be released within the carotid body by efferent nerves. However, the abundance of 5-HT in the carotid body does imply that it has some function in this organ and further studies seem warranted.

Local blood flow within the carotid body may be important in determining chemoreceptor discharge (Joels & Neil, 1963), so 5-HT could be more involved in regulating blood flow than in exerting a direct influence on the chemoreceptor cell-sensory nerve ending complex. Haemodynamic responses to 5-HT may be more subtle than simple changes in vascular tone. For example, 5-HT can increase vascular permeability, resulting in fluid-leakage into the perivascular spaces, and local haemoconcentration ('stasis') within the vessel (Majno & Palade, 1961; Majno *et al.*, 1961). It is doubtful whether such an action would be rapid enough, or sufficiently transient, to explain adequately any of the 5-HT effects we observed, but is indicative of the complex mechanisms that might mediate apparently simple effects of 5-HT. Histochemical studies in rats have confirmed the presence of 5-HT in carotid body type 1 cells, particularly those clustered around blood vessels (Grönblad *et al.*, 1983), so it is conceivable that 5-HT released from type 1 cells acts on 5-HT_2-receptors to alter vascular tone. Such changes in 5-HT output may have important consequences in certain pathophysiological states, such as hypertension (see Steele & Hinterberger, 1972).

In conclusion, 5-HT affects chemosensory discharge and at least two types of receptor appear to be involved in the responses evoked. Further studies with the new specific 5-HT agonists and antagonists, and utilizing neuropharmacological, ligand-binding and histochemical techniques, together with selective denervation of the carotid body, should establish where within this sensory organ these 5-HT receptors are located.

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