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CARDIOVASCULAR AND RESPIRATORY ACTIONS OF 5-HYDROXYTRYPTAMINE IN THE CAT

BY

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The respiratory and cardiovascular effects of 5-hydroxytryptamine (HT) in the cat have been described by several authors, but it is still not clear to what extent its effects are due to reflex or to direct actions.

HT causes a temporary arrest of breathing in the cat (Reid and Rand, 1951). Ginzel and Kottegoda (1954) observed that, when small doses (2–6 μg.) were injected into the carotid sinus region of the cat, there was a brief apnoea followed by stimulation of breathing; these changes were accompanied by a fall of blood pressure. Since denervation of the carotid sinus region abolished these effects they concluded that HT acts on either baro- or chemo-receptors. They also found that, after denervation of the carotid sinus, larger doses of the drug (50–100 μg.) once again caused similar respiratory and blood-pressure changes. From these and other observations they concluded that the effects produced by large doses of HT were due to actions on the central nervous system. While it is likely that, as with veratridine, large doses of HT cause cardiovascular and respiratory changes by central actions, other mechanisms also participate in the cardiovascular and respiratory effects produced by this substance.

Comroe, Van Lingen, Stroud, and Roncoroni (1953) observed that rapid intravenous injection of HT into cats caused one or all of the following effects: bradycardia, hypotension, apnoea, bronchial constriction, and an increase of right ventricular pressure. They showed that almost all these effects were, or might be, reflex; they concluded that the afferent nerve fibres were mainly in the vagi, and that the sensory receptors for the apnoea, bradycardia, and hypotension lie in the areas supplied by the pulmonary vessels and branches of the ascending aorta. They also suggested that HT might excite the coronary chemo-reflex (Bezold effect). Schneider and Yonkman (1953, 1954) reported that HT stimulates pulmonary stretch receptors, but admitted that some other type of sensory vagal nerve fibres might also be excited. Mott and Paintal (1953), in a small series of experiments, showed that the reflex apnoea caused by HT in the cat arose from sensory receptors lying between the great veins and the left atrium, and that it was abolished by cutting the vagi but not by cooling them to 2–3° C. Mott and Paintal (1953) and Paintal (1954) were unable to find any evidence that this substance stimulates or sensitizes pulmonary stretch, right or left atrial, or depressor, nerve endings. They concluded that the reflex apnoea caused by HT in the cat resembled that for the amidine compounds, and differed from that caused by veratridine; the latter has been shown to stimulate pulmonary stretch fibres (Meier, Bein, and Helmich, 1949; Dawes, Mott, and Widdicombe, 1951; Paintal, 1953). Hence there exists a difference of opinion regarding the site and mode of action of HT in causing reflex apnoea. The present investigation was undertaken in the hope of elucidating this question, and also with a view to analysing further reflex actions of HT in the cat.

METHODS

The respiratory and cardiovascular changes produced by intravenous injections of HT were studied in 13 cats (1.5–3.2 kg.). The animals were anaesthetized with chloralose (60 mg./kg.) and the respiratory movements recorded by the body plethysmograph (Dawes, Mott, and Widdicombe, 1951). Carotid blood pressure was recorded by a mercury manometer and injections were made into the jugular vein. The cervical vagi were cooled by the method described by Dawes *et al.* (1951). Both vagi were cooled simultaneously to the same temperature.

The effect of intravenous injections of HT on the blood flow in the hind legs was investigated in four cats (2.3–3.4 kg.). These animals were anaesthetized with pentobarbitone (30 mg./kg. intraperitoneally) and heparinized. The aorta was divided in the lower abdomen, a pump unit was introduced between the

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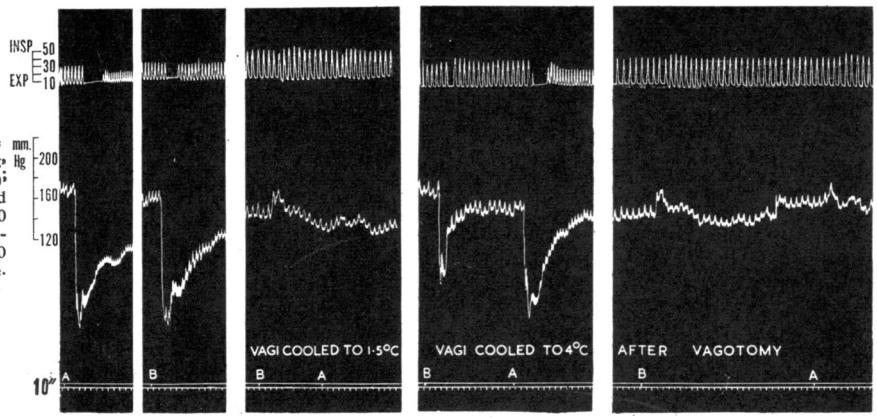

FIG. 1.—Cat. Chloralose 60 mg./kg. Top tracing, respiration (scale in ml.); lower tracing, carotid blood pressure. At A, 50 μg. 2α-naphthyl ethyl isothiourea i.v.; at B, 50 μg. 5-hydroxytryptamine. Time in 10 sec.

cut ends of the aorta (Vane, 1953) and the flow in the hind legs measured by the density flowmeter (Dawes, Mott, and Vane, 1953). The perfusion pressure, which was independent of the mean arterial pressure of the cat, was adjusted to correspond to the cat's blood pressure at the beginning of the experiment. Injections were made into the jugular vein and the carotid blood pressure was measured by a mercury manometer.

The effect of injecting small doses of HT into the heart was studied in 14 cats (2.1–4.6 kg.). They were anaesthetized with chloralose and heparinized. The chest was opened in the midline and the lungs ventilated artificially. Injections were made into the main left coronary artery using the method described by Dawes (1947). Blood pressure was recorded from the carotid artery by a mercury manometer and control injections of the same doses of the drug as were given into the coronary artery were made into a jugular vein.

In all experiments precautions were taken to eliminate errors from the tachyphylaxis which has been reported for HT (Reid, 1952; Freyburger, Graham, Rapport, Seay, Govier, Swoap, and Vander Brook, 1952; Gaddum, 1953; Ginzel and Kottegoda, 1953).

In three cats (2.7–3.8 kg.) anaesthetized with sodium pentobarbitone (32 mg./kg. i.p.) the vagi were divided to exclude reflex phenomena; the action potentials of single nerve fibres in the right vagus arising from slowly adapting pulmonary stretch endings were recorded using the method described by Widdicombe (1954a), but without filters. The intra-tracheal pressure was measured with a condenser manometer attached to one side arm of a tracheal cannula. The other two side tubes were attached to the output and input of a Palmer constant volume respiration pump which provided 20 inflations a minute. The chest was opened so that the pulmonary effects of artificial positive pressure respiration should not be interfered with by spontaneous movements of the chest wall. The tracheal pressure was displayed on one beam of a double-beam cathode-ray oscilloscope and the action potentials on the other. Injections of HT were made through the right brachial vein and the blood pressure was measured in the left femoral or iliac artery by a mercury manometer.

All doses of 5-hydroxytryptamine refer to the creatinine sulphate complex.

RESULTS

The Effect of Vagal Cooling and of Vagotomy on the Cardiovascular and Respiratory Actions of 5-Hydroxytryptamine

Circulation.—In all but 1 of the 13 cats used in these experiments 50–100 μg. HT intravenously caused a fall of blood pressure and bradycardia. Cooling the vagi to 1.5 or 2° C. abolished the effect in 7 cats (Fig. 1). In some of these the effects were not present when the vagi were cooled to 4° C., whereas in others cooling to 6–8° C. also abolished the effects. In 4 cats the effects were not abolished completely by cooling to 4–10° C. (Fig. 2). Out of 10 cats in which vagotomy was performed, in 8 the circulatory

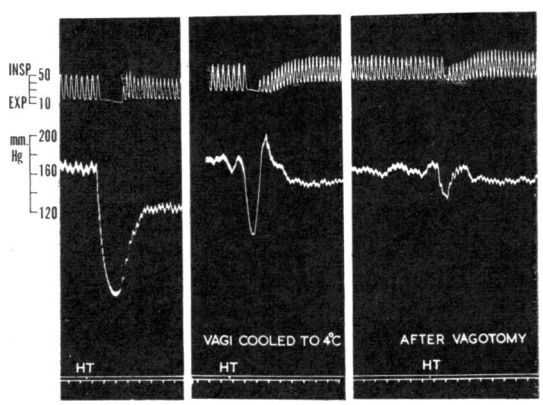

FIG. 2.—Cat. Chloralose, 60 mg./kg. Top tracing, respiration (scale in ml.); lower tracing, carotid blood pressure. At HT, 100 μg. 5-hydroxytryptamine i.v. Time in 10 sec.

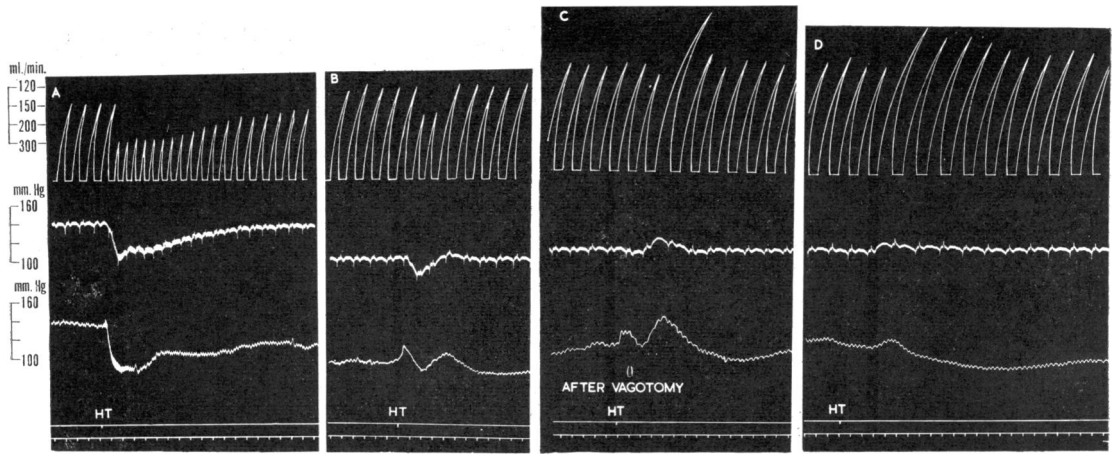

FIG. 3.—Cat. Sodium pentobarbitone, 32 mg./kg. Top tracing, rate of blood flow to perfused hind legs; middle tracing, perfusion pressure; bottom tracing, carotid blood pressure. At HT, 100 μg. 5-hydroxytryptamine intravenously in A, B, and C and intra-arterially in D. Between A and B, 4 mg. of atropine i.v. Between B and C, vagi cut. Time in 10 sec.

effects were abolished and in the other 2 there was still some effect remaining after vagotomy (Fig. 2).

Blood Flow in the Hind Legs.—In all 4 cats used in these experiments intravenous injections of 100–150 μg. HT caused an increase in blood flow in the hind legs during the fall of systemic blood pressure. This increase of flow was sometimes followed by a reduction. Comroe *et al.* (1953) believed that the hypotension caused by HT was mainly due to the bradycardia. In our experiments, after the animal was atropinized (1.5 mg./kg.) and the bradycardia prevented, intravenous HT still caused an increase in blood flow in the hind legs though to a less extent than before. Of the 3 experiments in which vagotomy was performed the increase in blood flow was abolished in 2 (Fig. 3), whereas in the third the effect was much reduced both in duration and in magnitude. After vagotomy, intravenous HT (100–150 μg.) often caused only a diminution of flow. When the same doses of the drug were injected into the inflow tube of the flowmeter—a procedure which amounted to direct intra-arterial injection into the hind legs—there was a large decrease in flow. This was probably due to the well-known direct vasoconstrictor action of HT which might normally counteract to some extent the reflex vasodilatation caused by the drug.

Intra-coronary Injection.—In all except 1 of the 14 cats used in this series of experiments intracoronary injection of 5–10 μg. caused a bradycardia and a fall of blood pressure (ranging from 20 to 90 mm. mercury). The same doses intravenously had either no effect on the blood pressure and heart rate or caused a small rise of pressure. Vagotomy was not performed in all these experiments, as the deterioration of the animal's condition—probably hastened by manipulation of the heart and by the effects of the drug—was such that in some experiments it was thought that any results obtained after vagotomy might be equivocal. Of 5 animals in which the vagi were cut

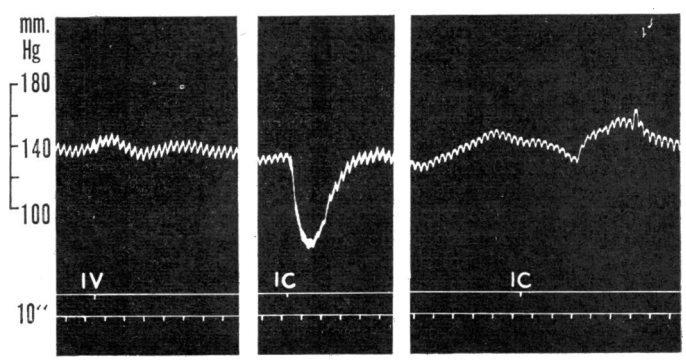

FIG. 4.—Cat. Chloralose, 60 mg./kg. Tracing of carotid blood pressure, 5 μg. HT at signal marks by intravenous (IV) or intra-coronary (IC) injection. Between the second and third portions of record, the vagi were cut. Time in 10 sec.

the hypotension and bradycardia were abolished in 4 (Fig. 4) and greatly reduced in the other. Similarly, in 5 of the 6 cats in which the vagi were cooled the effects were blocked at 6–8° C. In the remaining cat cooling to 6° C. reduced the effects but did not abolish them.

Respiration.—In 3 of the 13 cats used in these experiments the respiratory effects caused by injecting 50–100 μg. HT were not clear. In the remaining 10 cats the drug caused a period of apnoea which was often followed by a period of quickened breathing. In several of these animals the latter effect was accompanied by an increase in expiratory volume (functional residual air), as indicated by a rise in the whole record, very similar to that described by Dawes *et al.* (1951) for 2α-naphthyl ethyl isothiourea (see Fig. 2). The respiratory response was not altered by cooling the vagi to 8–10° C.; at temperatures between 8 and 2° C. there was a progressive diminution in the effect. In 5 of 6 cats cooling the vagi to 1.5 or 2° C. abolished the apnoea (Fig. 1) which could again be elicited by injecting HT after the nerves had regained body temperature. In 8 cats the injections were repeated after vagotomy. In 5 of these the apnoea was abolished, whereas in the other 3 there was a small residual effect (Fig. 2). In one cat in which the respiratory action persisted after vagotomy, cooling the vagi to 1° C. had also failed to abolish the apnoea completely.

Thus these observations confirm Mott and Paintal's (1953) conclusion that the effect of small doses of HT on breathing is not abolished by cooling the vagi to temperatures which are known to block conduction in pulmonary stretch fibres and the Hering-Breuer inhibitory reflex (Widdicombe, 1954a and b). Vagotomy always reduces, but does not always abolish, the apnoea ; there are, therefore, at least two mechanisms involved—one operating by means of afferent vagal nerve-fibres which are blocked by cooling below 2° C. (approximately), and the other by non-vagal mechanisms.

Pulmonary Stretch Receptors.—As the experiments described above showed that the respiratory response of the cat to HT was certainly not dominated by the Hering-Breuer reflex, it seemed desirable to repeat Schneider and Yonkman's experiments (1953, 1954) from which they concluded that HT stimulated the pulmonary stretch endings. It also seemed important to bear in mind the facts that the activity of pulmonary stretch endings can be modified by drugs known to cause changes in bronchial tone (Widdicombe, 1954c) and that HT can cause direct and reflex broncho-constriction (Comroe, Van Lingen, Stroud, and Roncoroni, 1953).

In three vagotomized cats, under artificial positive-pressure ventilation at constant volume, the action potentials from single pulmonary stretch endings,* and the intra-tracheal pressure, were recorded simultaneously. The intra-tracheal pressure and the frequency of discharge of the nerve fibre were measured at the peak of inflation ; in some experiments the frequency of discharge of the nerve fibre was also measured during deflation.

100–200 μg. HT (twice the quantity needed to produce the reflex effects described above) caused a rise of peak intra-tracheal pressure of from 0.6 to 2.0 mm. Hg (mean 16.5%, range 7–39%) in 14 of 15 tests. The increase in peak intra-tracheal pressure began to be evident on the first or second inflation, 2–7 sec. after the injection, but the maximum effect took 5–13 sec. to develop.

It was therefore clear that the injections of HT caused physical changes in the lung which need to be taken into account when attempting to interpret the effects of the injection on the activity of pulmonary stretch endings. The increase in peak intra-tracheal pressure at constant volume inflation, so regularly observed, is consistent with direct broncho-constriction by the drug.

The effects of the injections of HT on the activity of the pulmonary stretch endings varied. Six fibres showed a clear increase in frequency at the peaks of the inflations after the injection. The maximum increase of frequency (20–104%, mean 42%) usually occurred later (9–22 sec. after the injection) than the maximum pressure change. Three of the fibres showed activity throughout the respiratory cycle, and after the injection their activity increased during the *deflation* periods.

The initial response of six other fibres was a *fall* in frequency (mean 15%, range 6–38%) at the peak of inflations following the injection. In two fibres the peak frequency increased again, while the intra-tracheal pressure remained raised. One fibre which showed a *decrease* in frequency at the peaks of inflation after the injection nevertheless *increased* in frequency during the periods of deflation.

One of the remaining three records showed no rise of intra-tracheal pressure after the injection, and the effect on the peak frequency of the fibre was equivocal. The last two records both showed an increase of intra-tracheal pressure: on one the

* For the purposes of this paper a pulmonary stretch ending is one from which the action potentials increase in frequency during inflation of the lungs and decrease in frequency as they collapse. Endings from several different parts of the tracheo-bronchial tree and lungs may be included (Widdicombe, 1954a).

activity of the fibre showed no change, and on the other the result was equivocal.

It was noticed that aural judgments of the effect of an injection of HT on the activity of pulmonary stretch ending were unreliable, since the ear tended to confuse an increase of activity during the deflationary part of the cycle with a total increase of frequency; in such circumstances a decrease of frequency at the peak of inflation may not be appreciated.

Discussion

Circulation.—The cardiovascular effects of 5-hydroxytryptamine are complex, but it is clear that a proportion of the bradycardia and hypotension caused by the intravenous injection of 50–100 μg. is due to the coronary chemo-reflex (Dawes and Comroe, 1954), which can also be elicited by the intra-coronary injection of 5–10 μg. of HT. In most cats the cardiovascular effects of the intra-coronary injections were abolished by cooling the vagi to 6–8° C. At this temperature some, but not many, of the vagal *efferent* nerve fibres are still functional (Dawes, Mott, and Widdicombe, 1951), and if the *afferent* nerve fibres were intact there would be peripheral vasodilatation, which is shown, by the experiments on the hind legs, to contribute to the fall of blood pressure. The temperature at which the coronary chemo-reflex is abolished is a little lower than that reported for veratridine, and possibly lower than that for the amidines (Dawes, Mott, and Widdicombe, 1951), though the situation with these compounds is confused, as they also excite the pulmonary depressor chemo-reflex (Dawes and Comroe, 1953). HT also probably excites the pulmonary depressor reflex (Comroe, Van Lingen, Stroud, and Roncoroni, 1953), and this makes more difficult the precise definition of the coronary chemo-reflex with this compound. It is, however, clear, from the experiments reported in this paper, that the vagal temperatures required to abolish the whole of the vagal depressor effects are generally lower than those required to abolish only that portion due to the stimulation of receptors in the distribution of the coronary artery. This is indirect evidence in support of the stimulation of the pulmonary depressor reflex by HT in cats.

Respiration.—The results described in this paper confirm those of Mott and Paintal (1953). The apnoea caused by the injection of 50–100 μg. HT creatinine sulphate into cats can, in most instances, be shown to be reflex, since it is abolished by vagotomy or by cooling the vagi to 1–2° C. In some cats such injections cause an apnoea of non-vagal origin; but this, of course, does not exclude the co-existence of the reflex apnoea in the intact animal.

The experiments in which the vagi were cooled offer no support for the view that the pulmonary stretch endings are concerned to a significant extent in the reflex apnoea, since conduction along the fibres from such endings is abolished at 8°–10° C. (Dawes *et al.*, 1951) and cooling the vagi to this temperature does not reduce the respiratory response to HT.

The experiments also show that injection of 100–200 μg. HT (which is double the quantity required to produce the reflex apnoea) is sufficient to cause a mean increase of 16.5% in intra-tracheal pressure when the lungs are inflated at constant volume. This must mean that the injection causes a change in the compliance of the lungs. Two possible causes of this are broncho-constriction (Comroe, Van Lingen, Stroud, and Roncoroni), or a change in the amount of distribution of blood in the lungs —perhaps as a result of pulmonary vasoconstriction (Ginzel and Kottegoda, 1953). Whatever be the cause of the change of the physical response of the lung to constant volume inflation after HT, it is obvious that the effect of this drug on the activity of the pulmonary stretch fibres cannot be properly interpreted unless the disposition of the pulmonary stretch endings in the lung is known. The evidence on this point is somewhat indirect, though it has been suggested that these endings are in the bronchi, bronchioles or smaller air passages (Widdicombe, 1954c). If this view is correct, any drug which causes a change in bronchial tone may be expected to affect indirectly stretch receptors in the bronchial muscle. The effect on any individual receptor would depend on whether it was proximal or distal to the constricted segment, and whether it was in series or in parallel with the muscular elements. In the absence of histological or other evidence of the location of the endings further comment would be speculative, but it is at least possible that a drug could consistently cause broncho-constriction and yet have variable effects on the pulmonary stretch endings, according to their position relative to the constriction, and to the amount and distribution of the constriction.

If the twelve pulmonary stretch endings which gave clear-cut responses to injections of HT were a fair sample of such endings, it may be concluded that the total number of impulses due to non-reflex stimulation arriving at the respiratory centre would not be greatly increased, since the activity of six of the fibres increased whereas that of six

decreased. It is, of course, possible that the endings whose activity decreased are of a different type from those whose activity increased (Widdicombe, 1954a).

Veratridine is a substance which does cause reflex apnoea by stimulation of the pulmonary stretch endings (Dawes, Mott, and Widdicombe, 1951), but the response is much more intense than we have observed with 100–200 μg. of HT. The discharges of an ending stimulated with veratridine give rise to a whine in the loud-speaker within a very few seconds of the injection, and the latency of the reflex apnoea (as measured by the inhibition of phrenic motor neurones) is only 3.6–5.9 sec. (Jones, 1952). The response of a pulmonary stretch ending to HT takes 9–22 sec. to reach its peak and usually follows, rather than precedes, the increase in tracheal pressure. Presumably the smaller dose required to elicit the reflex respiratory response would cause even less disturbance of the pulmonary stretch endings.

Schneider and Yonkman (1953) have recorded stimulation of the pulmonary stretch endings with HT as intense as that observed by Dawes, Mott, and Widdicombe (1951) with veratridine, but the latency is still very long (5–15 sec.). It remains to be explained why a greater degree of stimulation was observed by Schneider and Yonkman (1953, 1954) than in the experiments described in this paper.

There was probably not any important difference in the depth of anaesthesia or in the dose of HT used in the two sets of experiments ; but it is conceivable that the degree of smooth muscle tone was not identical, and this may influence the bronchial responses (Widdicombe, 1954c). Inspection of Schneider and Yonkman's published records does, however, show that conspicuous bradycardia occurred. The presumption is that the second vagus was intact and that the coronary chemoreflex and the pulmonary depressor reflex (Dawes and Comroe, 1953) were active. The reflex respiratory affects of HT include broncho-constriction (Comroe, Van Lingen, Stroud, and Roncoroni, 1953). It is therefore likely that reflex as well as direct broncho-constriction occurred in Schneider and Yonkman's cats, and this might well have led to greater indirect stimulation of the pulmonary stretch endings. Paintal (1954) has recently suggested a similar interpretation of Schneider and Yonkman's observations. It is also possible that the reflex cardiac slowing might have caused an alteration in the volume or distribution of the blood in the lungs, but investigations on this point are lacking (Dawes and Comroe, 1953). Circulatory factors may be unimportant, since Schneider and Yonkman (1953) prevented the bradycardia with " Pendiomide " (Ciba 9295), but still obtained an increase in the activity of the pulmonary stretch endings. Schneider and Yonkman (1954) were unable to cause much increase of activity of pulmonary stretch endings by injecting HT in the rabbit, a species in which it is sometimes rather difficult to cause broncho-constriction (Banister, Fegler, and Hebb, 1950).

In conclusion, it seems certain that, in cats, HT causes a reflex apnoea mediated by small afferent nerve fibres, as yet unidentified, in the vagus. This apnoea closely resembles that caused by the amidines but is complicated by additional factors. It seems rather unlikely that HT stimulates directly the pulmonary stretch endings. However, the direct and reflex broncho-constriction which this compound is known to cause may contribute, by indirect stimulation of the pulmonary stretch endings, to the reflex apnoea observed, but not to such a degree as to permit any diminution in the total reflex respiratory response when the vagi are cooled to 8–10° C.

Summary

1. In 7 of 13 cats the bradycardia and fall of blood pressure caused by 50–100 μg. 5-hydroxytryptamine (HT) intravenously was abolished by cooling the vagi to 1.5 or 2° C. or by vagotomy. The hypotension was accompanied by increased blood flow through the hind legs, not abolished by atropine but greatly reduced or abolished by vagal section. HT thus causes bradycardia and a fall of blood pressure by stimulating afferent vagal fibres ; reflex peripheral vasodilatation contributes to the response.

2. Intra-coronary injection of 5–10 μg. HT caused bradycardia and hypotension (14/15 cats), usually abolished by cooling the vagi to 6–8° C. or by vagotomy. This accounts for part of the reflex cardiovascular effects caused by 50–100 μg. HT intravenously.

3. The apnoea elicited by HT (50–100 μg. i.v.) was usually abolished by cooling the vagi to 1.5 or 2° C. or by vagotomy and was therefore reflex ; but in some cats a brief apnoea was still produced by HT after vagotomy.

4. Intravenous injection of 100–200 μg. HT caused a small increase in peak intra-tracheal pressure in vagotomized cats given artificial positive-pressure ventilation at constant stroke volume, and was followed by changes in the rate of discharge of pulmonary stretch fibres. It is suggested that

these changes were secondary to direct bronchoconstriction caused by the HT.

5. The differences between the effects of HT on the activity of pulmonary stretch endings as described in this paper and as described by Schneider and Yonkman (1953, 1954) are discussed.

6. It is concluded that stimulation of pulmonary stretch endings is unlikely to play a dominant role in the production of reflex apnoea by the intravenous injection of 50–100 μg. HT in the cat.

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