J. Physiol. (1952) 118, 373–383

THE EFFECT OF A GANGLION-BLOCKING DRUG, HEXAMETHONIUM, ON THE RESPONSE OF THE CAT'S CAROTID BODY TO VARIOUS STIMULI

By W. W. DOUGLAS

From the National Institute for Medical Research, Mill Hill, London, N.W. 7

(*Received* 8 *May* 1952)

Anoxia sets up an afferent discharge in the sinus nerve, and there is evidence that the elements responding to oxygen lack are the glomus cells of the carotid body (De Castro, 1951). But little is known of the processes intervening between the detection of oxygen lack and its being signalled in the afferent nerve fibres. One possibility which has been suggested is that transmission on this sensory pathway is synaptic and akin to that occurring at ganglia on autonomic efferent pathways. This view, advanced by Euler, Liljestrand & Zotterman (1939), was based largely on the following considerations:

(1) Histological studies claiming the presence of ganglion cells in the carotid body.

(2) The fact that the carotid body can be stimulated by a group of substances known to stimulate autonomic ganglia, i.e. nicotine, lobeline, acetylcholine and KCl.

(3) Their belief that this group of substances acted at a point central to the oxygen sensitive elements of the carotid body: for, as they had shown, the stimulant action of these drugs was unaffected by ammonia which abolished the carotid body response to cyanide.

Recently, however, the possibility of ganglionic transmission has been called into question by Moe, Capo & Peralta (1948), who find that in the dog the carotid body responses to cyanide are uninfluenced by ganglion-blocking doses of tetraethylammonium.

The present paper reports the effect of the more potent ganglion-blocking compound, hexamethonium, on carotid body responses in the cat, the species which Euler *et al.* (1939) used in their experiments.

A preliminary account of the results now reported in detail was given to the Physiological Society in September 1951 (Douglas, 1951).

METHODS

The cats were anaesthetized with sodium pentobarbitone (nembutal). For induction, 40 mg/kg was given intraperitoneally: anaesthesia was maintained by giving small amounts intraperitoneally or intravenously from time to time.

The trachea was cannulated. Blood pressure was recorded from a femoral artery. Drugs were injected into a femoral vein.

Breathing was recorded in some animals by measuring diaphragm movements with the phrenograph described by de Candole, Douglas & Spencer (1950), in others by measuring tidal air with a small spirometer. In the latter case a closed circuit was used; oxygen was continuously fed in through a suitable valve, CO_2 absorbed by soda lime and movement of the air past the trachea maintained by valves or rotor pump.

Oxygen and nitrogen mixtures were prepared from cylinders of these gases. The composition of the mixture was determined by Haldane apparatus. By means of a valve arrangement attached to the trachea, the oxygen-poor mixture could be rapidly substituted for air.

The usual technique for carotid sinus-carotid body isolation, in which all the blood vessels coursing from the sinus and carotid body regions are tied, was not found to be satisfactory for the study of chemosensory reflexes. Probably as a result of stagnation of blood in the carotid body, a preparation of this sort frequently yielded only feeble chemoreceptor responses. In a number of experiments a technique was therefore adopted which permitted venous drainage from the carotid body. The lingual artery was tied and the maxillary artery cut between ligatures and reflected medially to facilitate exposure. The whole sinus and carotid body region was dissected clear of surrounding tissue and the various small vessels arising from the common carotid artery cut between ligatures. At this stage common carotid blood was flowing only by the vessels plunging deep from the sinus region. Amongst these vessels was a minute vein or veins which could be seen to arise from the carotid body and run to join the internal jugular or the large vein which runs towards the vertebral column. Care was taken not to occlude this venous drainage from the carotid body when the last mass ligature was tied. This ligature, which embraced the tenth, eleventh and twelfth nerves and the adjacent blood vessels, omitting only the ninth nerve and the sinus nerve, was placed as far cranially as possible, above the nodose and superior cervical ganglia and beyond the larger vein into which the vein or veins from the carotid body emptied. This larger vein was then cannulated and allowed to drain into a beaker. The volume of the venous effluent collected thus during the course of the experiment was usually so small that it was discarded; in one experiment, however, it was reinjected into the perfusing cat. The prepared carotid body region was now vascularly isolated by perfusing it with blood from a second heparinized cat. The blood was led from a femoral artery of this perfusing cat to the common carotid stump of the prepared region and returned to the femoral vein of the perfusing cat through another polythene tube leading from the stump of the maxillary artery. The tube leading blood to the perfused region was short and of small dead space. The return tube had a considerably greater dead space (about 4 ml.) and was fitted with a screw clamp and drop counter so that flow in the perfusion circuit could be maintained steady at about 12 ml./min. By maintaining the rate of flow of blood in the perfusion circuit constant despite any change in peripheral resistance or systemic blood pressure of the perfusing cat, it was ensured that tests of carotid body function were of comparable duration throughout the experiment. The arrangement also provided that any drug injected into the perfusion circuit proximal to the carotid body reached the perfusing cat only after a delay of about 20 sec. Any response of the perfused animal which occurred during this period could therefore be ascribed with confidence to an action of the drug in the perfused region.

Subsequent to the adoption of this venous drainage technique for the carotid body perfusion experiments now reported, Chungcharoen, Daly & Schweitzer (1952) published an anatomical study of the blood supply of the carotid body which had led them to conclude that damage to the venous drainage of the carotid body probably accounts for some of the experimental failures encountered in classical carotid sinus-carotid body perfusion experiments. The experience gained

in this present investigation has shown that the isolated carotid body region with venous drainage gives better results than the 'classical' preparation. However, one or two preparations with venous drainage were disappointing. In these the venous flow was practically absent. This probably resulted from excess manipulation during dissection, for the carotid body veins were observed to constrict vigorously to trauma.

RESULTS

Oxygen lack

The respiratory stimulant action of a brief period of anoxia is largely due to excitation of the chemosensory reflexes (Schmidt & Comroe, 1940); in the vagotomized cat the action is confined to the carotid bodies and provides a convenient test of their function. The respiratory response of vagotomized cats to breathing an oxygen-poor mixture (4·6–5·3% O_2 in N_2) for 30–50 sec was not lessened by large doses of hexamethonium (Fig. 1). Further experi-

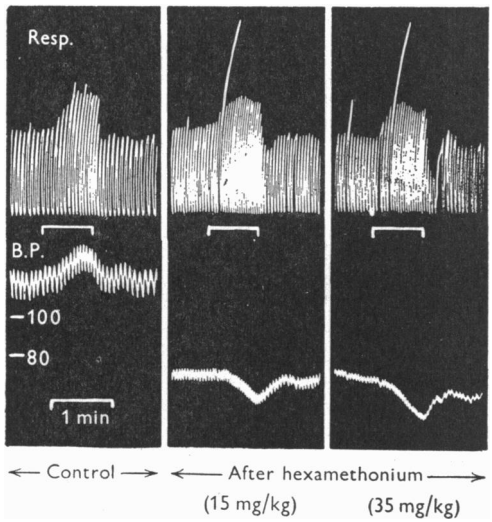

Fig. 1. Cat; nembutal; vagi cut. Diaphragm movements recorded by phrenograph. During the periods signalled the cat breathed 4·6% O_2 in N_2. Note that the respiratory response to this stimulus is undiminished by intravenous injection of hexamethonium. The lowered level of systemic blood pressure and the reversal of the anoxic pressor response following hexamethonium result from its blocking sympathetic ganglia.

ments, in which the carotid body was selectively exposed to oxygen lack and hexamethonium by perfusing the isolated carotid body region of one cat with blood from a second cat, corroborated this evidence.

In such experiments anoxia of the perfusing cat (caused by having it breathe an oxygen-poor mixture) stimulated respiration in the cat whose carotid body region was perfused purely by a reflex, for the effect was no longer obtainable

after cutting the sinus nerve. This reflex response to anoxia was not diminished when the carotid body was exposed to hexamethonium by giving 20 mg/kg of this drug to the perfusing cat (Fig. 2). It might be objected that in this experiment the respiratory stimulant effects obtained are due not to chemoreceptors but to a barosensory reflex caused by changes in the systemic blood pressure of the perfusing cat. This seems improbable, for before hexamethonium, anoxia of the perfusing cat would tend to raise its blood pressure and this pressor effect in the perfused carotid sinus would inhibit respiration rather than stimulate it, while after hexamethonium (which abolishes the pressor

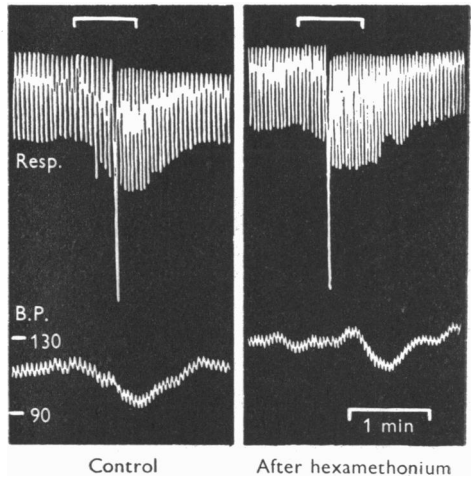

Fig. 2. Cat; nembutal; left sinus nerve and right vagus nerve cut; right carotid body region perfused by a second nembutalized cat. Tidal air recorded by spirometer (inspiration downwards). Blood pressure recorded from a femoral artery of perfused cat. During the periods signalled, the perfusing cat was made to breathe 5·8% O_2 in N_2. Note that the respiratory stimulation set up from the perfused carotid body is not diminished after the injection of hexamethonium (20 mg/kg) into the circulation of the perfusing cat.

effect of anoxia) the stimulant effect of anoxia developed, as can be seen, without there being any change in the systemic blood pressure of the perfused cat to indicate any barosensory effect.

Although cats given large doses of hexamethonium showed normal respiratory responses to brief periods of anoxia, they responded abnormally to longer periods (*c.* 80 sec) of oxygen lack. The response to anoxia was ill-sustained, and breathing failed sooner than in the absence of hexamethonium. This may have been due to the lowered systemic blood pressure and loss of the anoxic pressor response caused by sympathetic blockade, or to some direct action of hexamethonium on the respiratory centre.

Sodium cyanide

In the vagotomized cat, intravenously injected sodium cyanide gives a prompt, powerful stimulation of respiration due essentially to its excitant action on the carotid body (Schmidt & Comroe, 1940). This response, too, was undiminished after large doses of hexamethonium (Fig. 3). Nor was the effect of cyanide which was localized in perfusion experiments to the carotid body region lessened by giving hexamethonium (20 mg/kg) intravenously to the

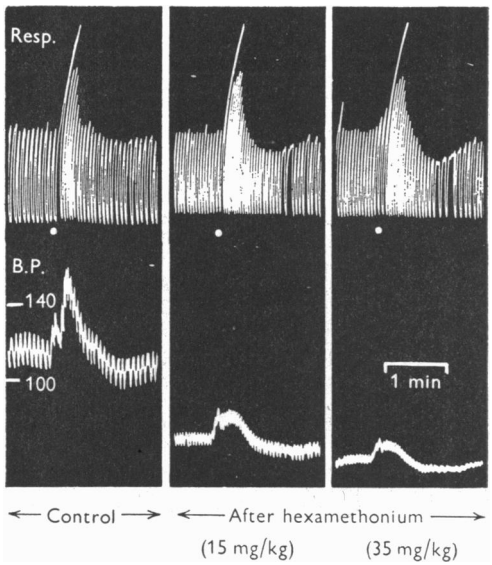

Fig. 3. Cat; nembutal; vagi cut. Diaphragm movements recorded by phrenograph. Blood pressure recorded from a femoral artery. At the points indicated on the respiratory trace 0·8 mg/kg NaCN was injected intravenously. Note the persistence of the respiratory response after hexamethonium.

perfusing cat (Fig. 5). In this experiment the respiratory stimulant effect of cyanide was clearly due to its exciting chemoreceptors. The response began immediately the cyanide was injected into the perfusion stream: the initial gasp and six or more of the succeeding deepened breaths occurred before the cyanide had reached the perfusing cat (see methods). Once in the circulation of the perfusing cat, this comparatively small dose of cyanide (200 μg) is unlikely to have caused a change in systemic blood pressure sufficient to influence the established chemosensory response by any barosensory reflex.

Nicotine, lobeline and acetylcholine

Suitable doses of nicotine, lobeline or acetylcholine, given intravenously, each caused a short, vigorous burst of overbreathing. After hexamethonium this response was profoundly reduced or abolished. This result was obtained

with all three substances, although in each experiment the response to anoxia or cyanide was unimpaired. A typical result with acetylcholine and lobeline is shown in Fig. 4. Perfusion experiments showed that hexamethonium blocked the stimulant action of these substances at the carotid body region. Thus, nicotine, acetylcholine or lobeline, each of which stimulated respiration when injected into the blood stream perfusing the carotid body, all failed to stimulate or did so only feebly when injected after hexamethonium had been given to the perfusing cat. An experiment with acetylcholine is illustrated in Fig. 5. As was the case for the cyanide effect shown in this same figure, the

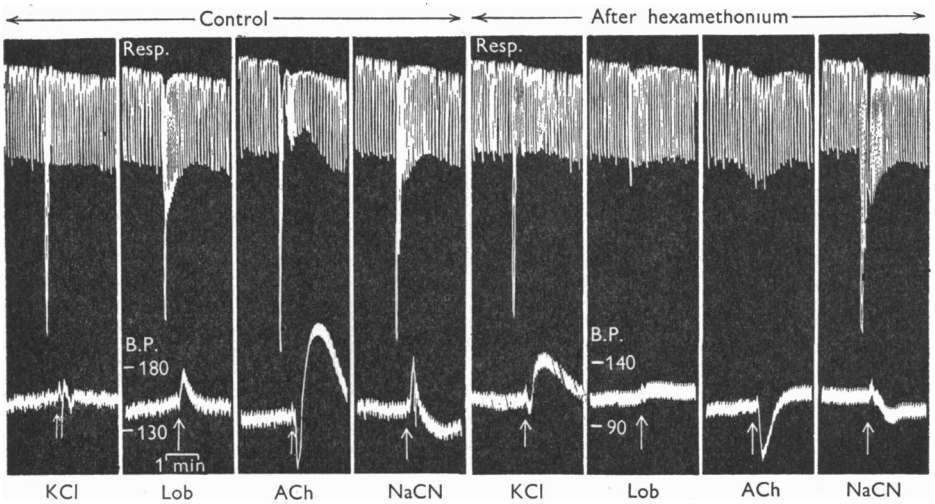

Fig. 4. Cat; nembutal; vagi cut; atropine 1 mg/kg. Records: tidal air (spirometer); blood pressure. Intravenous injection of KCl (2·4 mg), lobeline (0·5 mg), acetylcholine (1 mg) and sodium cyanide (1 mg) before and after hexamethonium (15 mg/kg). Note that hexamethonium suppresses the respiratory responses to lobeline and acetylcholine but not those to KCl and NaCN. The presence of sympathetic block after hexamethonium is well illustrated by the abolition of the pressor response to acetylcholine.

response to acetylcholine injected into the perfusion stream was established and already diminishing before the acetylcholine reached the circulation of the perfusing cat, and the respiratory response recorded must therefore be attributed to carotid chemoreceptors. Since the perfusing cat is atropinized (1 mg/kg), the subsequent entry of 100 μg acetylcholine into its circulation is unlikely to have caused any marked change in systemic blood pressure which might modify greatly the course of this chemosensory response.

Potassium

Intravenous injection of 1·2–2·4 mg KCl (1·2% in Ringer-Locke solution) was found to stimulate respiration. In some cats the action was feeble, in others well pronounced, but it was always more fleeting than the response to

nicotine, lobeline or acetylcholine. It was a reflex effect and could be abolished by cutting the vagi and sinus nerves. Stimulation of respiration could also be obtained by injecting a smaller amount of KCl into a common carotid artery through a cannula inserted into the thyroid artery. In such experiments the external carotid distal to the lingual artery was tied to prevent access of potassium chloride to the brain by this route and, by slowing the flow, to prolong its local action. This method gave a longer-lasting effect which was abolished by dividing the sinus nerve.

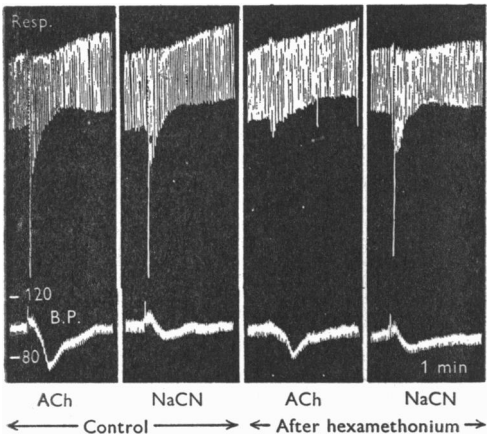

Fig. 5

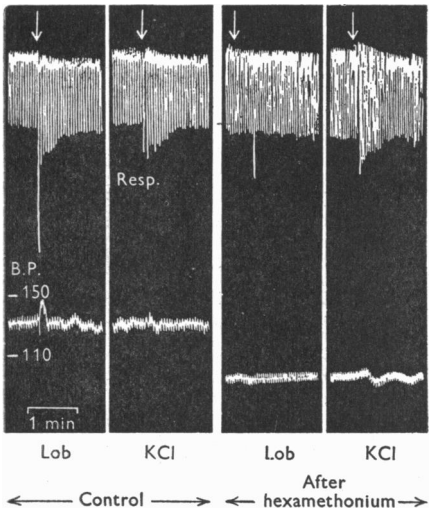

Fig. 6

Fig. 5. Cat; nembutal; left sinus nerve and right vagus nerve cut; right carotid body region perfused by a second nembutalized cat which has also been atropinized (1 mg/kg). Records: tidal air (spirometer); blood pressure of perfused cat. Injection of acetylcholine (0·1 mg) and sodium cyanide (0·2 mg) into the perfused region through the thyroid artery. Note that after injecting hexamethonium (20 mg/kg) into the perfusing cat, the response to acetylcholine is much reduced but that to sodium cyanide unaffected.

Fig. 6. Cat; nembutal; vagi cut. Records: tidal air (spirometer); blood pressure. Injections of lobeline (20 μg) and KCl (120 μg) into the right carotid artery (external carotid artery tied) before and after hexamethonium (15 mg/kg). Note that the respiratory stimulant action of lobeline is no longer present after hexamethonium but that the response to KCl persists.

It was found that intravenous injection of hexamethonium (15 mg/kg) was without effect on the respiratory response to KCl given by the intravenous or intracarotid routes (Figs. 4 and 6).

DISCUSSION

The results show that the ganglion-blocking compound, hexamethonium, affects the response of the carotid body to certain stimuli but not to others. It abolishes the stimulant actions of lobeline, nicotine and acetylcholine, but

does not interfere with those of oxygen lack and cyanide. Any theory of transmission of carotid body responses must take these facts into consideration.

If the chemosensory afferent pathway from the oxygen-sensitive elements in the carotid body were to involve synaptic transmission akin to that occurring in the autonomic nervous system, then one would expect hexamethonium to block the respiratory stimulant effect of oxygen lack. However, hexamethonium in doses much greater than those causing profound block of sympathetic and parasympathetic ganglia (Paton & Zaimis, 1951) failed to exert any apparent action on the carotid body responses to anoxia or cyanide. It is therefore difficult to accept the view that transmission of these responses is synaptic, unless one assumes that the postulated synapse has a resistance to hexamethonium so great that it escapes entirely the effects of doses blocking even the more resistant ganglia, such as those of the cardiac vagus.

If, as Euler *et al.* (1939), have concluded, the site of action of acetylcholine and other substances with nicotine-like action were a synapse on the afferent pathway from the oxygen-sensitive cells, then it would be exceedingly difficult to explain how this synapse can be rendered unresponsive to these substances by hexamethonium and yet transmit impulses from the oxygen-sensitive cells, especially as acetylcholine is suggested to be the chemical transmitter (Liljestrand, 1951).

The results obtained with hexamethonium thus support neither the hypothesis of ganglionic transmission nor the assumption that substances with nicotine-like action act at a point central to oxygen lack. It would appear that the pathway from the oxygen-sensitive elements of the cat's carotid body runs to the respiratory centre uninterrupted by any ganglion-like synapse. This, in fact, is the conclusion which De Castro (1951) has arrived at as a result of his histological studies.

The mechanism of the carotid body stimulant action of nicotine-like drugs

If the presence of a ganglion-like relay on the afferent pathway signalling oxygen lack is to be discounted, an explanation of the carotid body stimulating action of nicotine, lobeline and acetylcholine other than that offered by Euler *et al.* (1939) must be sought.

It is possible that drugs with nicotine-like action stimulate afferents arising in the carotid body region quite distinct from those normally signalling oxygen lack. But this seems unlikely, for the reflexes initiated by these drugs and those set up by oxygen lack are very similar. It seems more probable that all these stimuli activate the same afferent group. If this is the case, then it is necessary to explain how substances such as acetylcholine and nicotine, whose action is generally considered to be confined to ganglion-like structures, excite a sensory pathway devoid of such elements. An answer seems to be provided by the evidence that nicotine and acetylcholine can excite nerve

endings in the cat's skin where no ganglia occur (Coon & Rothman, 1940; Brown & Gray, 1948) and that this response of the skin nerves can be blocked by nicotine (Brown & Gray, 1948) or by hexamethonium (W. W. Douglas & J. A. B. Gray, unpublished). Such evidence makes it unnecessary to postulate the presence of a 'synapse' to account for the carotid body stimulant effect of substances acting like nicotine or to explain the action of hexamethonium in preventing this effect. The actions of these drugs may well be on the oxygen-sensitive glomus cells or their afferent nerves. It is possible, however, that there are anatomically distinct peripheral elements, some responding to oxygen lack, and others to drugs with nicotine-like action, for a single afferent fibre may serve several glomus cells (De Castro, 1951) and glomus cells differ histologically one from the other (Koelle, 1951; Kock, 1950). But whatever may be the morphological basis of the sensitivity to acetylcholine, nicotine or lobeline, this sensitivity, being selectively abolished by hexamethonium, must be considered a property additional to the normal function of signalling oxygen want, rather than a consequence of the processes involved in this normal function. This behaviour of the carotid body is quite analogous to that of the sensory nerve fibres of the skin; these also maintain their normal sensory function, i.e. response to touch, after their sensitivity to nicotine and acetylcholine has been abolished (Brown & Gray, 1948; W. W. Douglas & J. A. B. Gray, unpublished).

Euler *et al.* (1939), assuming K^+ to be a 'synaptotropic agent', considered the sensitivity of the chemosensory reflex to K^+ as further evidence of ganglia on the carotid body afferent path mechanisms. There is, however, even less ground for considering potassium to have a specific site of action at ganglia than for holding compounds with nicotine-like action to act exclusively in this way. Brown & MacIntosh (1939) have in fact shown that potassium stimulates nerve fibres in continuity.

The persistence of the carotid body response to oxygen lack or to K^+ in the presence of hexamethonium shows that the effect of hexamethonium on the carotid body response to nicotine, acetylcholine or lobeline is not due to an unspecific local anaesthetic action. Hexamethonium probably blocks the action of these drugs by virtue of some structural similarity with their sensory excitant groupings.

The possible role of acetylcholine in peripheral chemosensory function

Schweitzer & Wright (1938), without envisaging synaptic transmission, suggested that acetylcholine is somehow involved in the processes which set up activity in the chemosensory fibres of the sinus nerve. Although their view was to some extent based on the stimulant action of injected acetylcholine—an action which has less significance now that it is known that it can be abolished without interfering with normal function—it may yet prove to be correct. Some effects of eserine and atropine on the carotid body (Kaindl &

Werner, 1948; Liljestrand, 1951) support their theory. On this more general view of acetylcholine involvement in chemoreceptor function—a view which does not attribute to acetylcholine the role of chemical transmitter at some ganglion-like synapse—the action of hexamethonium sheds little light. De Castro's (1951) morphological interpretation of the cat's carotid body mechanisms shows the afferent chemosensory neurone to have origin *within* the glomus cell. If this be the site of endogenous acetylcholine action, it may well escape the influence of hexamethonium. Hexamethonium does not readily penetrate the cellular membrane: in the body it distributes itself in the extracellular space (W. D. M. Paton, personal communication). Similarly, acetylcholine is unlikely to stimulate the intracellular part of the afferent neurone. Abolition of the response to injected acetylcholine such as occurs with hexamethonium is therefore not incompatible with intracellular acetylcholine transmission of anoxial responses.

SUMMARY

1. The effect of the ganglion-blocking compound hexamethonium on the carotid body afferent mechanisms has been studied in the cat under pentobarbitone anaesthesia.

2. The carotid body responses to nicotine, lobeline and acetylcholine are greatly reduced or abolished by hexamethonium.

3. Carotid body responses to anoxia or cyanide, however, are unaffected by hexamethonium, even in doses causing profound block of autonomic ganglia.

4. The stimulant action of KCl persists after hexamethonium.

5. The selective abolition of the response to the drugs with nicotine-like effects indicates that these do not act at a synapse on the chemosensory afferent path. An alternative explanation is offered for their stimulant action and that of K^+.

6. It is concluded that the afferent impulses set up in the carotid body by oxygen lack are not transmitted through synapses similar to those of ganglia in the autonomic nervous system.

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