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THE EFFECT OF HEXAMETHONIUM ON THE CAROTID CHEMORECEPTOR RESPONSE TO NICOTINE AND CYANIDE

BY

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The literature concerning the effects of ganglionic blocking agents on the chemoreceptors is reviewed. Hexamethonium blocks the respiratory response to intracarotid injections of small doses of nicotine in dogs anaesthetized with chloralose, but it does not block the response to sodium cyanide.

There has been considerable controversy about the effects of hexamethonium and other ganglionic blocking agents on the chemoreceptor responses of the carotid and aortic bodies to various stimuli (see Table 1). In the course of our investigations of the actions of sympathomimetic amines on the carotid chemoreceptors (Byck & Pelikan, 1960), we have had occasion to use hexamethonium as a pharmacological tool in attempting to define the mode of action of certain drugs including that of sodium cyanide and nicotine. The following experiments were designed to resolve the controversy regarding hexamethonium chemoreceptor block.

METHODS

Thirteen dogs weighing from 7.4 to 14.2 kg were anaesthetized by intravenous injection of a 10% solution of chloralose in polyethylene glycol 200 (80 mg/kg) 1 hr after subcutaneous injection of morphine sulphate (1 mg/kg).

Cannulae were inserted into the trachea, a femoral artery, a femoral vein, and both superior thyroid arteries (the latter directed toward the common carotid artery). Since dogs given hexamethonium intravenously maintain a constant blood level of the drug after nephrectomy (Monge, Corcoran, DelGreco & Page, 1954), the renal artery and vein were ligated bilaterally before each experiment. The procedure was carried out through a midline abdominal incision and the wound was closed with sutures.

Respiration was recorded quantitatively by a modification of a bag-box system which separates inspired and expired gas (Donald & Christie, 1949). The animal inspired through a valve from a thin-walled, 50-litre meteorological balloon which was enclosed in an air-tight 60-gallon metal drum; this provided an inexpensive bag-box system which required filling infrequently. The animal expired air into the space around the bag through a valve. In the original method, Donald & Christie measured tidal vol. by recording the excursions of a spirometer connected to the inside of the box. In these experiments a sensitive strain gauge was substituted for the spirometer and the pressure fluctuations during inspiration and expira-

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tion were related to vol. of gas breathed. This method separates inspired and expired gas so that gases of different compositions may be breathed, but still permits a quantitative measure of tidal vol. The response time of this system was found to be less than 0.1 sec and the accuracy of measurement of a single breath to be approximately 2%. The tidal vol. of a single breath or respiratory min vol. over a period of time could thus be determined with ease. Arterial blood pressure was recorded from a femoral artery. Chemoreceptor stimulant drugs were injected in slightly greater than threshold concentrations into the common carotid blood stream via the cannulae in the superior thyroid arteries; 0.1 ml. of drug was flushed in with 1.0 ml. of 0.9% NaCl. Hexamethonium was given intravenously (50 to 200 mg total dose). The exact time of injection was marked on the record by a micro-switch triggered by the injection syringe. Injections were made into alternate arteries, allowing a total time lapse of at least 15 min between injections into any one artery. All intracarotid doses are total amounts.

In order to demonstrate that the respiratory response was due to chemoreceptor stimulation, all drugs were tested after section of the sinus nerve or infiltration of the area of the carotid bifurcation with 2% lignocaine.

Control injections. A control injection of 0.9% NaCl was given between all drug injections in exactly the same manner as the drugs; this never produced a significant response. Intravenous injections of polyethylene glycol 200 in 2 dogs anaesthetized with 1% chloralose dissolved in warm saline did not diminish the respiratory response to chemoreceptor stimulants.

All drugs were prepared as fresh stock solutions in 0.9% NaCl. Nicotine was used as the base and hexamethonium as the chloride.

Polyglycol E-200 was provided through the courtesy of the Dow Chemical Company, Midland, Michigan. The chloralose used in these experiments was "Pur alpha chloralose," Kuhlmann Produits R.A.L., Paris.

RESULTS

Measurement of records. The usual response to intracarotid injection of small doses of chemoreceptor stimulants was a single deep breath followed by an almost immediate return to normal respiration (Figs. 1 and 3). Larger doses caused an intense hyperpnoea of variable duration. The response to small doses was related to the dose given; in a series of 10 dogs in which 64 doses of nicotine (0.2 to 1.0 μg) were given, an increase in dose resulted in an increased response in 90% of the tests. Since the dose-response curve was not linear over a larger range of doses, we used a minimal effective dose as a standard of comparison. The ED30 was chosen as a reliable estimate because it was found that this response was comfortably above the normal variations in respiration.

The maximal effect on tidal vol. was limited by the maximal inspiratory capacity of the dog. Supramaximal doses did not produce consistent maximal inspiratory efforts in the first 5 sec and so the data could not be plotted as a difference from a known maximal response. Plotting the responses in this way (using the largest breath the dog made with any dose as a maximum) did not reduce the variability seen in the responses to a single dose given at different times in the experiment. Transformation of the data in other ways such as dose against absolute increase in tidal vol. or dose against vol. of maximal breath in the response similarly did not reduce the variability. Since diminished control tidal vol. seemed to be a function of depth of anaesthesia and was associated with diminished responsiveness of the animal, percentage increase of tidal vol. above control tidal vol. was used as the measure of response.

As a guide to the significance of the blocking effects, the ED30 of nicotine (10 dogs) was 0.28 μg. From this figure, shown graphically on a log. scale in Fig. 2,

an ED30 which is greater by a statistically significant margin can be estimated. A significant block was considered to have been demonstrated when the ED30 after ganglionic blockade was more than 3 standard deviations from the control ED30. Equivalent figures were calculated for sodium cyanide and established as criteria for block.

Nicotine. In low doses there was a clear relationship between the dose used and the increase in ventilation. This response was abolished by section or block of Hering's nerve.

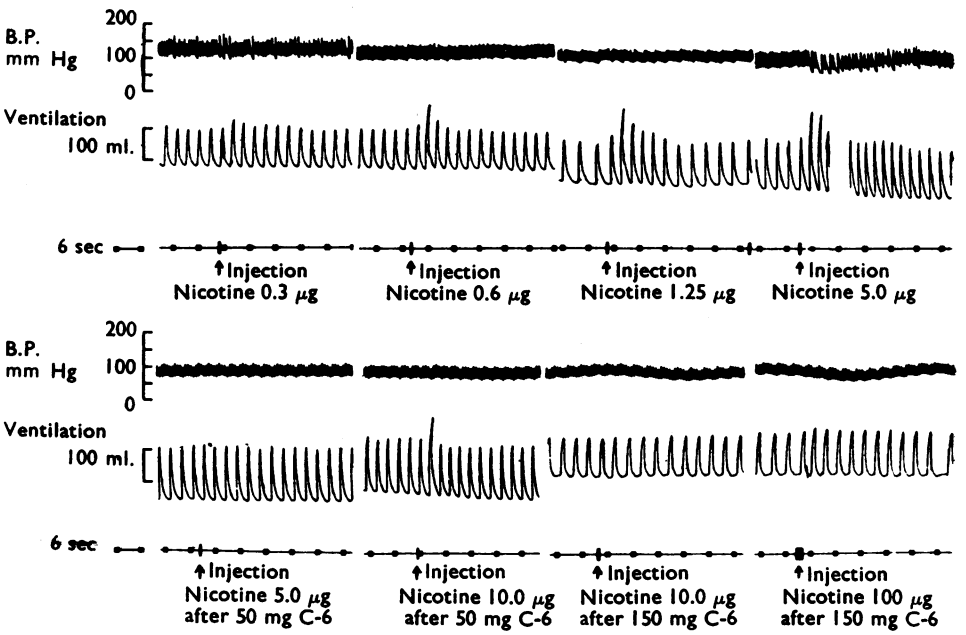

Fig. 1. Blood pressure and respiration responses to graded intracarotid doses of nicotine in a dog under chloralose anaesthesia before and after hexamethonium (C-6) given intravenously.

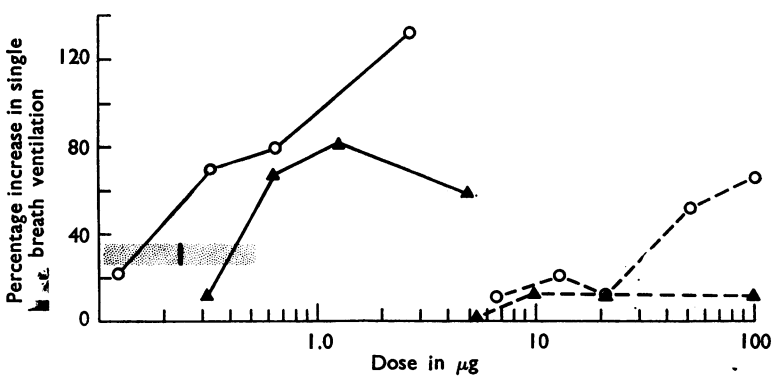

Fig. 2. Dose-effect (respiration) curves for graded intracarotid doses of nicotine in two experiments. Shaded area shows one standard deviation on each side of the mean ED30 for 10 dogs. Expt. 1: before ▲——▲, after ▲ - - ▲. Expt. 2: before O——O, after O - - O (50 mg C-6).

In Fig. 1 responses to nicotine before and after administration of hexamethonium are illustrated. There is a blockade of previously effective small doses. Larger doses of nicotine still caused a response. In all dogs tested 50 mg of hexamethonium abolished the response of intracarotid doses of nicotine up to 6.25 μg. At doses between 6.25 and 100 μg, the hexamethonium block was not consistently obtained. The response to 100 μg of nicotine could not be blocked even when an almost lethal dose of hexamethonium was administered; it was therefore concluded that hexamethonium causes a competitive block of the chemoreceptor response to nicotine. The shift in the dose-effect curve in 2 experiments is shown in Fig. 2.

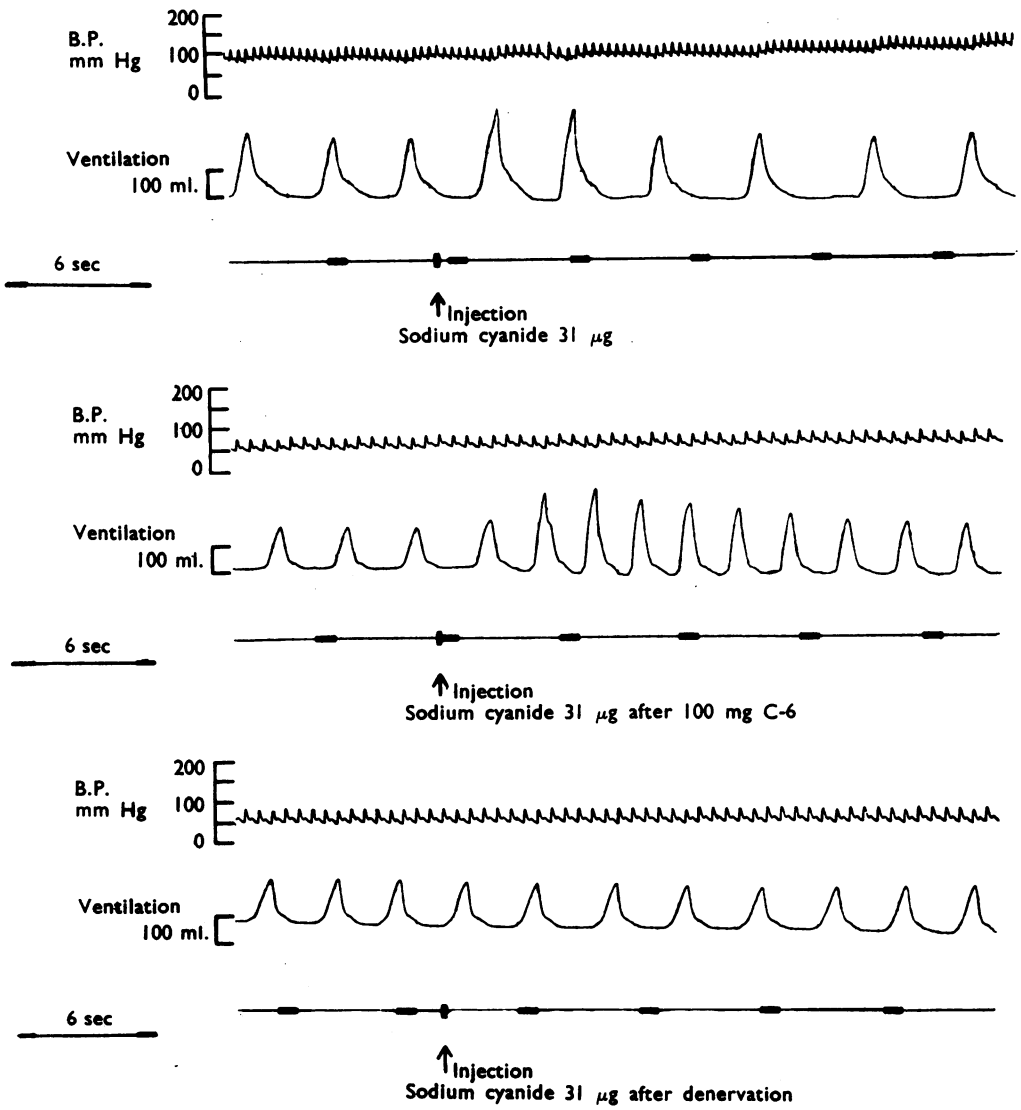

Fig. 3. Blood pressure and respiration responses to intracarotid injections of sodium cyanide in a dog under chloralose anaesthesia before and after hexamethonium and denervation.

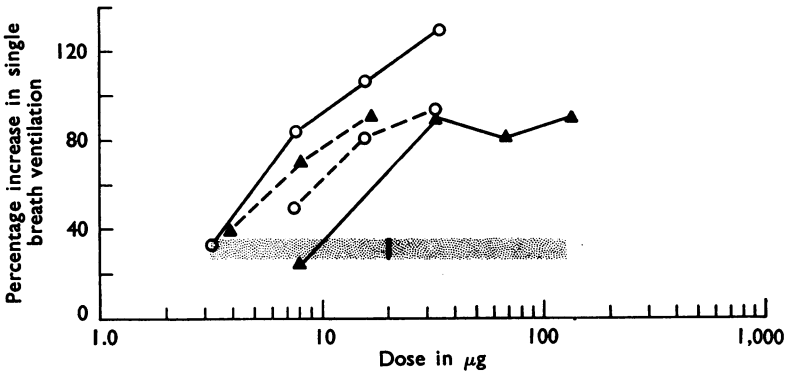

Fig. 4. Dose-effect (respiration) curves for graded intracarotid doses of sodium cyanide in 2 experiments. Shaded area shows one standard deviation on each side of the mean ED30 for 10 dogs. Expt. 1: before ▲——▲, after ▲ - - ▲. Expt. 2: before O——O, after O - - O (50 mg C-6).

Sodium cyanide. Again a clear relation was found between the dose and the increase in response over the dose range used. There was no evidence of diminution of response to cyanide after any dose of hexamethonium (Fig. 3). In some animals there appeared to be a potentiation of the response to cyanide after hexamethonium. This potentiation might well be due to the increased excitability of the chemoreceptors secondary to a decrease in blood pressure (Floyd & Neil, 1952). It also might be analogous to the increased duration of response to cyanide found in studies recording nerve impulses in the sinus nerve of the cat (Dontas & Nickerson, 1956). The shift in the dose-effect curve is shown in Fig. 4.

DISCUSSION

We believe that the controversy regarding the effect of hexamethonium on the carotid chemoreceptors is due to disagreement on three points:

1. What is the proper criterion for chemoreceptor stimulation? Are changes in respiration and blood pressure too non-specific to allow conclusions about the effects of drugs on the chemoreceptors?

2. Can a competitive block be found if supramaximal doses of stimulant drugs are used and, conversely, is there any meaning to a block found after massive intra-arterial doses of blocking agents?

3. Must a response be inhibited almost completely to demonstrate block (Heymans, Delaunois, Martini & Janssen, 1953), or is a significant shift of the dose-response curve sufficient evidence of inhibition or block?

First let us discuss the method of recording and measurement. Recording of respiratory parameters is a direct measurement of the end result of chemoreceptor action. This is desirable because it provides a measure of the total respiratory effect of the injected drug. If one could eliminate the other variables which might affect respiration, this would be the ideal method of evaluating chemoreceptor stimulant activity. To utilize the advantages of the respiratory measurement, one must be able to minimize interfering factors, detect subtle differences in volume or

rate, and finally, make a quantitative estimate of the respiratory response in a significant way.

A quantitative estimate of the respiratory response is possible using several different parameters. The use of the bag-box system allows calculations of tidal volume of single breaths as well as respiratory minute volume over any selected interval of time.

Recordings from the sinus nerve can certainly detect changes in afferent activity, but there may be uncertainty about the receptors stimulated, and whether the impulses are directed to the respiratory centre or to other regions. The method lends itself to assay, although this possibility has not yet been fully explored.

The second point of contention has been dosages, both of the chemoreceptor stimulants and the blocking agents. If one is to obtain significant results, one should use doses of drugs that could conceivably be blocked by competitive agents such as hexamethonium. The doses used by Heymans *et al.* (1953), which produced a marked hyperpnoea and where no dose-effect relationship could be seen, are clearly supramaximal. An equal imbalance is seen in the use of large intra-arterial doses of blocking agents. As Heymans & Neil (1958) comment, " We are somewhat dubious about the effects of huge amounts of these drugs given locally into the carotid circulation. We feel that the *p*H of the solutions used and the bromide or iodide ion of the compounds may be complicating the responses seen in these conditions."

The final element of design is the criterion of block. Considering the mode of action of hexamethonium, one would not expect a complete block of any compound *a priori* but, rather, would expect a significant shift of the dose-effect curve or a significant diminution of the response to individual doses. Heymans *et al.* (1953) feel that complete inhibition of the respiratory response is necessary to demonstrate blockade.

In our experiments we have attempted to meet all of these objections as follows. (1) A direct and quantitative measure of response which fits a dose-effect relationship was used (Byck, 1957). (2) The doses of both chemoreceptor stimulant drugs and blocking agents were varied. The doses of the chemoreceptor stimulants varied from just threshold (for example, 0.3 μg nicotine) to supramaximal (for example, 100 μg nicotine). The doses of the blocking agent used, although high, were not excessive and were given by the intravenous rather than the intra-arterial route. (3) The presence of a block was demonstrated by the use of dose-effect curves as well as by the illustration of the inhibited response.

Different preparations, animals and anaesthetics have been used in the various investigations cited in Table 1. Since our results are diametrically opposed to two of these, namely, Heymans *et al.* (1953) and Boelaert (1948), it is necessary to examine their experiments in some detail. Both authors measured the respiratory responses of dogs under chloralose anaesthesia. Heymans *et al.* (1953) used both hexamethonium and tetraethylammonium. They used respiratory min vol. as a measure of effect, and, although they emphasize the importance of the dose-effect relationship as a basis for drawing conclusions, they gave no data on varying responses to varying doses. Further, in their tracing in this paper on p. 215 there is two to three times the respiratory response to the same dose of lobeline and acetylcholine

TABLE 1

THE EFFECT OF GANGLIONIC BLOCKING AGENTS ON CHEMORECEPTOR RESPONSES

Authors	Animal	Preparation and recording	Anaesthesia	Blocking agent and dose	Route of administration of blocking agent
A. Block of nicotine-like drugs and no block of cyanide or anoxia					
Dontas & Nickerson (1956)	Cat	Record from sinus nerve	Chloralose	Hexamethonium 1.0 mg total dose	Close intra-arterial injection
Douglas (1952)	Cat	Crossed circulation, record of respiration	Pentobarbitone sodium	Hexamethonium 15 mg/kg	Intravenous
Moe *et al.* (1948)	Dog	Record of respiration	Thiopentone +barbitone	Tetraethylammonium 20 to 40 mg/kg/hr	Continuous intravenous infusion
This paper	Dog	Record of respiration	Chloralose	Hexamethonium 50 to 200 mg (5 to 20 mg/kg)	Intravenous
B. Block or decreased response to both nicotine-like drugs and cyanide or anoxia					
Landgren *et al.* (1952)	Cat	Record from sinus nerve	Chloralose Urethane	Tetraethylammonium 100 mg	Close intra-arterial injection
				200 mg	Intravenous
Gollwitzer-Meier (1953)	Cat	Record from sinus nerve	Chloralose	Hexamethonium 1.0 mg total dose	Close intra-arterial injection
C. Block of neither nicotine-like drugs nor cyanide or anoxia					
Heymans *et al.* (1953)	Dog	Record of respiration	Chloralose	Hexamethonium 2 to 5 mg	Locally into conjunctival sac
				Tetraethylammonium 10 mg/kg	Intravenous
Boelaert (1948) (As quoted by Heymans and Neil, 1958)	Dog	Not stated	Chloralose	Tetraethylammonium—dose not stated	Not stated

before hexamethonium as after hexamethonium, whereas the response to cyanide did not change appreciably. Further, because of the relatively short duration of action of ganglionic blocking agents, it is necessary to have some method of keeping constant blood levels of the drugs in order to compare blocking activity. Moe, Capo & Peralta (1948) used a continuous intravenous infusion of tetraethylammonium. In the present experiments the dogs were "nephrectomized." Heymans *et al.* (1953), however, had no method of keeping a constant level of blocking agent in their experiments, and they do not record or comment on the duration of blockade achieved with a single injection technique; therefore, even a greater degree of block may have been present at the time of highest tissue concentration.

We cannot comment on the work of Boelaert (1948) because of the lack of specific information in the paper as published.

The experiments of Landgren, Liljestrand & Zotterman (1952) and Gollwitzer-Meier & Witzleb (1953) are open to the criticism previously quoted from Heymans & Neil (1958), that very concentrated intra-arterial doses of blocking agents may have non-specific actions. Indeed, when Landgren *et al.* (1952) used intravenous doses of blocking agents, they too demonstrated a diminished response to nicotine-like but not to anoxic stimulation.

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