THE EFFECT OF NEMBUTAL® ANESTHESIA ON THE CARDIAC RESPONSE TO ACETYLCHOLINE * †

HARRY A. TEITELBAUM, M.D. AND W. HORSLEY GANTT, M.D.

Baltimore, Maryland

WHILE carrying out experiments, by means of an apparatus designed by us (1), to determine whether the effect of acetylcholine on the heart could be conditioned, we (2) observed that the cardiac reaction to acetylcholine in the unanesthetized dog differed from that in the nembutal anesthetized animal. This observation led to further experimentation, the results of which are reported below. Also, the value of our apparatus for other than conditional reflex studies is thus indicated.

In line with our observations, Emmelin (3), Schutz (4, 5) and Fernando (6) have observed that the barbiturates influence the action of acetylcholine. Although Ellis and Weiss (7) reported cardiac acceleration but no bradycardia following intravenous administration of acetylcholine in man, Goldenberg and Rothberger (8), Noth, Essex and Barnes (9), and Greenberg and Lambeth (10) described cardiac inhibition followed by acceleration, as we (2) did, as the effect of acetylcholine administration. That the action of acetylcholine on the heart is peripheral rather than through the central nervous system, has been established by Hoffman, Middleton and Essex (11), McNamara, Krop and McKay (12), Greenberg and Lambeth (10), Heymans and Bennati (13) and McDowell.

METHOD

By means of our (1) apparatus, we are able to inject drugs intravenously into trained, unanesthetized dogs, isolated in a soundproof camera. This is accomplished by inserting a large bore needle into the radial vein, and passing a fine plastic tube through the needle into the vein. The needle is then withdrawn, leaving the plastic tube in place. The tube in the vein is connected to the lower end of a glass cylinder containing the drug solution. The upper end of the cylinder is continuous with plastic tubing that extends through the camera wall to the outside. Layered over the drug solution in the cylinder is heavy mineral oil that fills the cylinder as well as the plastic tubing extending to the outside of the camera. In this way we have a continuous fluid system of mineral oil layered over the drug solution. By injecting a

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quantity of oil into the tubing from outside the camera, an equal quantity of drug solution is displaced into the venous circulation.

By means of the apparatus described above, acetylcholine chloride in 1 or 2 per cent solution was injected intravenously into 5 dogs, usually in 0.4 cc. doses. Smaller (0.2 cc.) and larger (0.6 cc.) doses were used at times, as indicated. The drug was studied in some dogs in the unanesthetized and anesthetized states on the same day. After a series of injections was given to the unanesthetized dog, nembutal was administered intravenously, 60 mg. per 5 pounds, and then another series of injections was given. In other instances the observations in the unanesthetized and anesthetized states, respectively, were made on different days.

The heart rate was recorded by means of a brush development oscillograph and amplifier, on paper calibrated to permit the reading of heart beats per second. In counting the heart rate, the time intervals between beats were considered as units, so that it was possible to count fractions of beats per second.

RESULTS

Effect of Nembutal on Heart Rate. The heart rate of dogs in the unanesthetized state was found to be slower and more variable than in the anesthetized state. For instance in one dog (Dog 1) the preinjection control rates for five injections varied between 66 and 222 beats per minute, when the rate was calculated for one second intervals. The respective averages of the five controls, calculated for one second intervals, varied between 95 and 126 (fig. 1). After anesthesia, the preinjection control heart rates for five injections varied between 156 and 180 beats per minute, with the respective averages varying between 162 and 170 (fig. 1), calculated for intervals of one second.

Table 1 contains data on the 5 dogs studied. In the first two columns, the average heart rates are compared in the unanesthetized and anesthetized states during respective fifteen second intervals, immediately preceding the injection of acetylcholine. They are, therefore, marked "control." Comparison of columns 1 and 2, table 1, reveals that the average heart rates in the anesthetized state show absolute increases over those in the unanesthetized states of 16, 50, 72, 47 and 51 beats per minute, respectively. The respective percentage increases in heart rate are 13, 42, 118, 38 and 52.

Effect of Acetylcholine on Heart Rate. That acetylcholine slows the heart rate and then accelerates it is well illustrated in figure 1. The inhibition is not constant and usually precedes the acceleration, which is a constant response. In one dog 200 injections of acetylcholine caused acceleration each time, but inhibition only 159 times. In another dog 200 injections that caused acceleration, caused inhibition only 50 times. In some instances a number of fast beats occurred

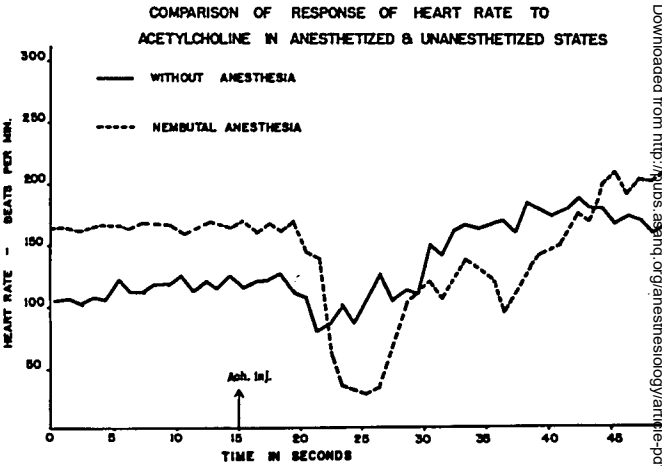

FIG. 1. Comparison of response of heart rate to acetylcholine in anesthetized and unanesthetized states. The effect of nembutal anesthesia in increasing the heart rate is illustrated in the preinjection, control graphs. The rate per minute is plotted at one second intervals.

before the inhibition set in. This observation agrees in essence with McDowall's (14) finding that in a few hearts the slowing is preceded by a few forcible contractions.

The use of acetylcholine in graduated doses showed that small doses caused acceleration alone as a rule, while larger doses produced inconstant temporary inhibition followed by acceleration of the heart

TABLE 1

EFFECT OF NEMBUTAL ANESTHESIA ON THE REACTION OF THE HEART RATE TO ACETYLCHOLINE IN 5 DOGS*

Dog	Control, Average for 15 Seconds		Rate at Greatest Inhibition due to Acetylcholine		Inhibition (of Control), per cent		Rate at Greatest Acceleration due to Acetylcholine		Acceleration (of Control), per cent	
	Unan.	An.	Unan.	An.	Unan.	An.	Unan.	An.	Unan.	An.
1	119	135	64	32	46.2	76.3	278	267	133	97
2	119	169	91	33	21.9	77.5	210	256	84.3	53.9
3	61	132	48	25	23	80.9	179	183	192.5	44.2
4	122	169	68	45	40.9	72.6	251	215	118.3	26.7
5	97	148	60	12	37.6	92.1	232	170	156.7	17.1

* The figures are averages of a number of injections in each instance, as follows: Dog 1, Unan.—10; An—12. Dog 2, Unan.—12; An.—11. Dog 3, Unan.—8; An.—13. Dog 4, Unan.—13; An.—8. Dog 5, Unan.—5; An.—12.

rate. In the anesthetized state, one dog (Dog 1) showed the responses recorded in table 2A. Here 0.1 cc. of 1 per cent acetylcholine caused marked acceleration but no inhibition; with increasing doses of drug, the inhibition appeared and became more marked. A relative relationship between dosage and inhibition is illustrated in table 2B. In these tables it can be seen that complete cardiac arrest is usually of longer duration with increasing dosage.

Although in table 2A, there appears to be greater acceleration of heart rate with increasing dosage of drug, this finding is not constant

TABLE 2

EFFECT OF GRADED DOSES OF 1 PER CENT ACETYLCHOLINE CHOLRIDE ON THE HEART RATE PER MINUTE OF 2 NEMBUTAL ANESTHETIZED DOGS

Dose, cc.	Control, Average for 15 Seconds	Inhibited Rate	Duration of Complete Standstill in Seconds	Accelerated Rate
		A. Dog 1		
0.1	125	138	0	198
0.2	122	42	1	258
0.4	120	18	2	272
		B. Dog 5*		
0.2	169	24	>1	180
0.2	150	12	>4	150
0.4	180	12	>4, >1	180
0.4	120	12	>6	174
0.4	118	6	>8	174
0.4	114	12	>4	138
0.6	174	6	>7, >1	180
0.6	162	6	>7, >3	168
0.6	120	12	>4	138
0.6	118	7	>6, >1	194

* In B (Dog 5), the two figures in column 4 indicate intervals of complete cardiac standstill occurring in immediate succession, with only one heart beat between them.

within the range of doses used, as is evident in table 2B. Here the higher doses of drug do not show greater cardiac acceleration, but do show relatively poor acceleration in some instances.

Effect of Nembutal on Cardiac Inhibition Due to Acetylcholine. The latent period for maximal cardiac inhibition due to acetylcholine varied between seven and sixteen seconds in 2 dogs in the unanesthetized state, and between six and eighteen seconds while under nembutal anesthesia.

The accentuating effect of nembutal anesthesia on cardiac inhib-

tion attributable to acetylcholine is strikingly evident in the greater duration of complete cardiac arrest in the anesthetized state. Of forty-one injections in 5 dogs, without anesthesia, cardiac arrest for longer than one second (less than two seconds) occurred only six times, while in the other thirty-five instances the cessation of heart action lasted less than one second. Under nembutal anesthesia, cardiac arrest for less than one second occurred only three times. The respective durations of complete cardiac standstill resulting from the other injections were: over one second, 5 injections; over two seconds, 17; over three seconds, 11; over four seconds, 6; over six seconds, 2; over eight seconds, 1.

The graph (fig. 1) shows the greater quantitative slowing of the heart rate by acetylcholine in the nembutal anesthetized state, as compared to that in the unanesthetized state. Since the control heart rate is faster in the anesthetized state, the inhibition due to acetylcholine, when compared to that in the unanesthetized state, is even more striking. The same differences in cardiac inhibition in the anesthetized and unanesthetized states, respectively, are apparent in table 1. For instance in one dog (Dog 1, table 1), the diminution in heart rate caused by acetylcholine was from 119 per minute to 64 per minute (46.2 per cent) in the unanesthetized state, although it fell from 135 per minute to 32 per minute (76.5 per cent) in the anesthetized state. In the anesthetized state there was a greater absolute diminution in heart rate attributable to acetylcholine, as is evident in comparing columns 3 and 4, table 1. This greater absolute diminution in heart rate in the 5 dogs, respectively, is 32 per minute, 58 per minute, 23 per minute, 23 per minute, and 48 per minute. The greater percentage diminution in heart rate from respective controls, due to acetylcholine in the anesthetized state as compared to that in the unanesthetized state, is evident in columns 5 and 6, table 1.

Effect of Nembutal on Cardiac Acceleration Due to Acetylcholine. The time interval between drug injection and maximal acceleration, as well as that between maximal inhibition and maximal acceleration, showed considerable variation. Nembutal anesthesia had no significant effect on these time intervals.

There was no significant relationship between the dose of acetylcholine and the degree of acceleration in the dosage range employed. It is likely that with smaller amounts of acetylcholine, the threshold for acceleration would become evident. It is apparent in table 1, columns 7 and 8, that nembutal anesthesia does not have any specific effect on the cardiac acceleration caused by acetylcholine. In 2 dogs (Dogs 2 and 3), the acceleration was greater in the anesthetized state, with that in Dog 3 being insignificant—only 4 per minute. In the other 3 dogs the acceleration was greater in the unanesthetized state, with that in Dog 1 being insignificant, only 11 per minute. The percentage in-

creases in heart rate due to acetylcholine over their respective controls was greater in the unanesthetized state in all 5 dogs. This is attributable, at least in part, to the lower control heart rates. Thus, although the graph in figure 1 shows that the acceleration due to acetylcholine rises to greater heights in the anesthetized than in the unanesthetized state, this is characteristic only of this animal, and not a constant feature.

The return to the preinjection, control cardiac rate, following acceleration, occurred in a markedly variable period of time, one to ninety-one seconds. This was not significantly influenced by anesthesia, for under the latter, this time interval varied from one to 126 seconds.

DISCUSSION

Our observations confirm the inhibitory and accelerator effects of acetylcholine on the heart rate. We agree with McDowall's (14) findings that small doses of acetylcholine cause stimulation only, although with larger doses, inhibition also appeared. Greenberg and Lambeth (10) reported that minimal doses of acetylcholine produced only cardiac acceleration.

Although the data reported here do not shed any light on the site of action of this drug, we (15) have completed experiments that show that the cardiac reaction to acetylcholine does not become conditioned, and is thus a peripheral rather than a central process. With drugs that act peripherally, as pilocarpine on salivation, cardiac reaction fails to become conditioned, as shown by Kleitman (16), Mulinos and Lieb (17), and Finch (18), while with drugs that act centrally, as morphine on salivation, cardiac reaction can be conditioned, as reported by Collins and Tatum (19), Kleitman and Crisler (20), Mulinos and Lieb (17). Therefore, the failure of the cardiac reaction to acetylcholine to become conditioned indicates a peripheral action of this drug. This would agree with the findings of Hoffman *et al.* (11), McNamara *et al.* (12), Greenberg and Lambeth (10), Heymans and Bennati (13), and McDowall (14).

Several studies are available on the mechanisms involved in the action of acetylcholine on the heart. McDowall (14) and Hoffman *et al.* (11) found that atropine blocks the inhibitory effect of acetylcholine and accentuates the accelerator effect, while ergotamine, curare and nicotine abolish the accelerator action. Hoffman *et al.* (11), McNamara *et al.* (12) and Greenberg and Lambeth (10) found evidence that an epinephrine-like substance and epinephrine mediate the cardiac acceleration produced by acetylcholine. This observation gains further support in that acetylcholine does mediate epinephrine seretion owing to splanchnic nerve stimulation, as shown by Feldberg, Minz and Tzudzimura (21). The cardiac acceleration is also due to the nicotinic action of acetylcholine, and involves an intracardiac, ganglionic syn-

aptic process, as it is blocked by "tetraethylammonium" according to Heymans and Benatti (13), as well as by nicotine according to Hoffman *et al.* (11) and McNamara *et al.* (12).

Our observation of the increase in heart rate with nembutal anesthesia agrees with that of Hafkesbring and MacCalmont (22). Hoffman *et al.* (11) found that atropine blocks the cardiac inhibition produced by acetylcholine, thus indicating that this inhibition is the result of the "muscarine" action of acetylcholine, which is counteracted by cholinesterase. Thus, the action of nembutal in accelerating the heart would suggest that this acceleration is not an anticholinesterase effect, for such an effect would theoretically enhance the "muscarine" action of acetylcholine and slow the heart. However, the nembutal effect of accentuating the cardiac inhibition caused by acetylcholine does suggest an anticholinesterase action by that anesthetic. Although Schutz (4, 5) reported anticholinesterase activity by barbiturates, this was observed only after prolonged administration of barbiturates. In our study, nembutal effects were observed with single, narcotic doses of this drug. Furthermore, although Emmelin (3) did observe increased sensitivity to acetylcholine, attributable to luminal, this effect was completely independent of any possible cholinesterase activity. The problem is further complicated by Fernando's (6) observation that barbiturates block cardiac inhibition in amplitude due to acetylcholine.

The more likely explanation for the action of nembutal would be its effect on cardiovascular homeostatic mechanisms. In this respect, Greenberg and Lambeth (10) reported that, in the unanesthetized dog, "A minimal dose of acetylcholine produced a transient depressor effect accompanied by an increased heart rate," while a large dose caused "temporary heart arrest, followed by an increased heart rate for ten to fifteen seconds until the blood pressure rose to above resting blood pressure, after which there was a slowing of heart rate to less than normal, lasting for several minutes." In the same dog, following cardiac denervation, the same minimal dose of acetylcholine caused a "transient vasodepressor effect and no change in heart rate," while the larger dose caused a "temporary heart arrest, followed by a gradual rise in pressure to slightly above normal and an increase in heart rate which began later and was much greater than in the normal dog." Thus, the cardiac acceleration associated with the vasodepression is dependent on neural reflexes, as denervation abolishes it. The delayed tachycardia, however, which occurs twenty-five seconds after cardiac arrest, is due to epinephrine released reflexly and directly, by the action of acetylcholine on the adrenal gland.

Goodman and Gilman (23) pointed out that in barbiturate anesthesia the carotid sinus mechanism is depressed, and cardiovascular reflexes are diminished. This could delay the reflex cardiac acceleration associated with hypotension, for this acceleration is apparently dependent on intact cardiac innervation, as shown by Greenberg and Lambeth

(10). With this delay in reflex cardiac acceleration under nembutal anesthesia, the cardiac arrest due to acetylcholine would be prolonged. The later acceleration due to epinephrine, occurring twenty-five seconds after cardiac arrest, is not affected by nembutal anesthesia.

SUMMARY

Acetylcholine chloride, in the unanesthetized dog, was observed to have an inhibitory, followed by an accelerating, effect on the heart rate.

Nembutal anesthesia stabilized and increased the heart rate.

Acetylcholine had a more marked inhibitory effect on the heart rate in the nembutal anesthetized state than in the unanesthetized state of the dogs studied. Although our studies showed that the acceleration caused by acetylcholine was not influenced by nembutal anesthesia, there are indications that the early phase of this acceleration is inhibited by nembutal, thus contributing to the accentuation of cardiac arrest due to acetylcholine.

The mechanisms involved in these observations are discussed.

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