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EFFECT OF HEMICHOLINIUM ON THE RELEASE OF AUTONOMIC MEDIATORS IN THE SINOATRIAL NODE

BY

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For the past four years, we have used a technique of selective stimulation of intramyocardial autonomic nerve fibres in a variety of isolated heart tissue preparations. The technique is " selective " in that electrical stimuli of appropriate characteristics, when applied directly to the myocardium, are capable of exciting intramyocardial autonomic nerve fibres without exciting the myocardium *per se*. Selective stimulation of intramural autonomic nerve fibres is not without precedent, and has been reported for *vas deferens* (Birmingham & Wilson, 1963) and trachea (Carlyle, 1964) as well as for heart (Ursillo, 1958; Lewartowski, 1963; Vincenzi & West, 1963).

The chronotropic and inotropic responses produced by applying appropriate electrical stimuli to cardiac tissue depend on the release of acetylcholine and noradrenaline (Amory & West, 1962; Vincenzi & West, 1963). We have used the term " electro-release " to describe this general phenomenon in order to differentiate it from what might be termed " chemo-release " (Bhagat & Shideman, 1963; Hall, 1963). It should be noted that the stimuli used in this study, although applied directly to the sinoatrial node, were subthreshold for the specialized cardiac tissue of the sinoatrial node, but were suprathreshold for intranodal autonomic nerve fibres.

Direct electrical stimulation of the isolated spontaneously beating sinoatrial node elicits a biphasic chronotropic response (West, 1961; Amory & West, 1962) indicating that fibres of both divisions of the autonomic nervous system are excited by such a procedure. The initial slowing of spontaneous rate (cholinergic electro-release response) is potentiated by physostigmine and blocked by atropine. The secondary increase in spontaneous rate (adrenergic electro-release response) is potentiated by cocaine and blocked by reserpine, bretylium or guanethidine, or by a β-receptor blocking agent such as dichloroisoprenaline (Amory & West, 1962). The spontaneously beating sinoatrial node preparation thus provides an isolated system in which one may easily observe the function of postganglionic nerve fibres simultaneously subjected to identical conditions. Therefore, we considered this a desirable system in which to test the provocative hypothesis of Burn & Rand (1959).

The Burn & Rand hypothesis states that a cholinergic link is involved in the release of noradrenaline from fibres which have hitherto been considered " adrenergic." Hemicholinium No. 3, a drug which blocks the synthesis of acetylcholine (MacIntosh, 1961), has been cited as providing the most direct evidence for this hypothesis (Burn & Rand,

1962). Such evidence rests on reports, such as that of Chang & Rand (1960), that hemicholinium blocks the action of sympathetic nerves. Using the double-innervated system described above, we have found no evidence of an inhibitory effect of hemicholinium on the action of postganglionic accelerator nerve endings.

METHODS

Rabbits of either sex weighing 1.7 to 2.3 kg were stunned and killed by bleeding. Cats weighing 1.5 to 2.5 kg were anaesthetized with ether before being bled to death. Sinoatrial nodes with contiguous right atria were isolated and mounted in a Plexiglas bath maintained at $35 \pm 0.5°$ C. Diastolic tension was set at 1 g and contractile tension was monitored using a Grass (FT 03) strain gauge. Preparations were continuously perfused with approximately 2 ml./min of Ringer-Locke solution of the following composition (mmoles/l.): NaCl 145, KCl 5.4, $CaCl_2$ 2.2, $NaHCO_3$ 11.9, and dextrose 11.0. The nutrient solution was gassed with 95% oxygen and 5% carbon dioxide. Preparations were allowed to equilibrate for 60 to 90 min before treatment with hemicholinium.

Bursts of rectangular stimuli (10 sec duration, 50 shocks/sec, 10 V positive) were applied directly to the sinoatrial node every 200 sec throughout each experiment unless otherwise stated. Pulse duration ranged from 0.05 to 0.5 msec in various experiments. The stimulating electrodes consisted of a 3 mm diameter silver plate facing the epicardial surface of the sinoatrial node and a 1 mm diameter silver wire (insulated to the tip) applied to the endocardial sinoatrial node surface.

Spontaneously propagated action potentials were recorded from the atrial myocardium using a bipolar silver-wire electrode. The amplified action potential triggered a device which reset a linear voltage ramp generator calibrated for time intervals. The ramp generator thus served as a meter of the interval between each spontaneous beat. Changes in spontaneous beat interval induced by intranodal nerve stimulation served as a measure of sympathetic and parasympathetic nerve function. Three measurements of spontaneous beat interval were used to characterize each chronotropic response. These were: the steady state interval just before stimulus application; the maximal interval after the beginning of nerve stimulation; and the minimal interval during or following nerve stimulation. For a given chronotropic response, the difference between the steady state interval and the maximal interval is directly related to the cholinergic electro-release response. Likewise, the difference between the steady state interval and the minimal interval is directly related to the adrenergic electro-release response.

The drugs used were hemicholinium (Aldrich Chemical Co.), atropine sulphate and acetylcholine bromide. Hemicholinium and atropine were dissolved in the nutrient solution and applied in continuous perfusion as described. Acetylcholine was added directly to the bath to test sinoatrial node sensitivity to the cholinergic transmitter.

RESULTS

Results and records presented here were all derived from preparations obtained from rabbits. Three preparations from cats provided qualitatively identical results.

Hemicholinium did not produce any obvious changes in the contractile tension developed by the right atrial myocardium. Results are not given, however, because of the uncertain meaning of drug-induced changes in contractile tension of preparations whose rate is not controlled (Koch-Weser & Blinks, 1963).

Hemicholinium (5×10^{-5} g/ml.) had little effect on the steady state spontaneous beat interval (Fig. 1). At 5×10^{-4} g/ml., hemicholinium produced a slight reduction in the steady state interval (increase in spontaneous rate) in non-atropinized preparations (Fig. 3).

In concentrations ranging from 10^{-5} to 5×10^{-4} g/ml., hemicholinium caused progressive block of the cholinergic electro-release response. Fig. 1 illustrates interval meter records of the chronotropic response before and after treatment with 5×10^{-5} g/ml. of hemi-

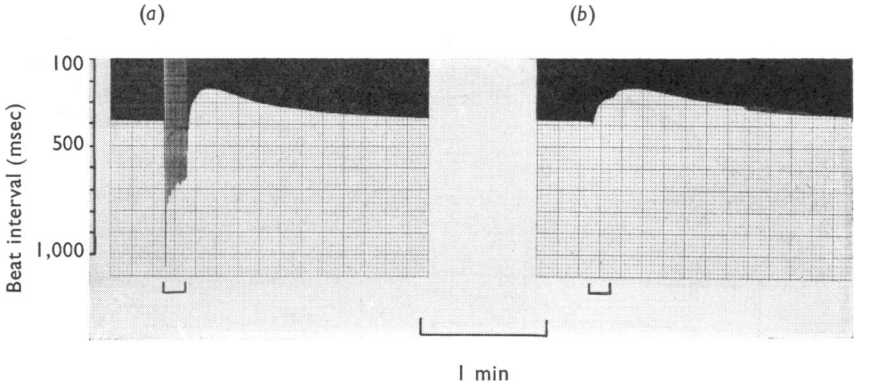

Fig. 1. Effect of hemicholinium on the chronotropic response to intranodal nerve stimulation. Interval meter records of: (*a*) control chronotropic response; (*b*) chronotropic response after perfusion with hemicholinium (5×10^{-5} g/ml.) for 1 hr. Horizontal bars indicate the periods of intranodal nerve stimulation. The inverted scale indicates spontaneous beat interval in msec. Beat interval is read from the lower border of the dark portion of the record.

cholinium for 1 hr. With that concentration, the block had a relatively slow onset (Fig. 2). The block caused by hemicholinium was not due to an atropine-like action. In several experiments acetylcholine was added directly to the bath. This produced a negative chronotropic response at a time when the cholinergic electro-release response was blocked. Also, in several experiments in which stimuli were not applied during the initial perfusion with hemicholinium, no block developed. Hence, nerve stimulation was necessary for

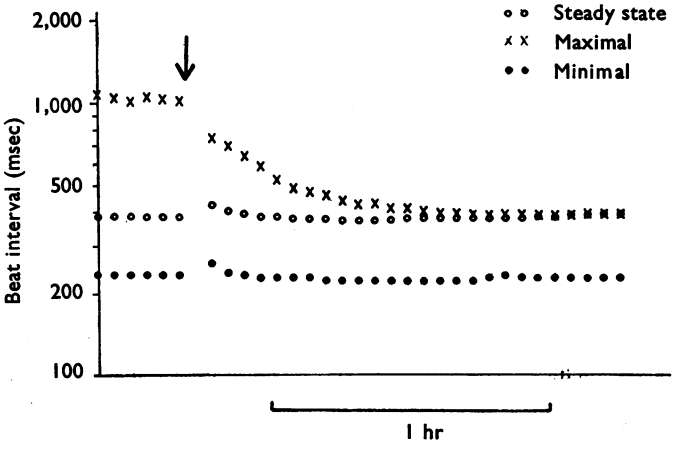

Fig. 2. Time course of the action of hemicholinium. Semilog plot of steady state, maximal and minimal beat intervals before and during perfusion with hemicholinium (5×10^{-5} g/ml., continuously present from the arrow). At any given time, the three intervals serve to characterize a single chronotropic response to intranodal nerve stimulation. Steady state interval is the spontaneous beat interval just before intranodal nerve stimulation. Maximal and minimal intervals are the maximal and minimal spontaneous beat intervals, respectively, which occur during or after intranodal nerve stimulation. In the presence of hemicholinium, the maximal interval (a measure of the cholinergic electro-release response) was progressively reduced while the minimal interval (a measure of the adrenergic electro-release response) remained unchanged.

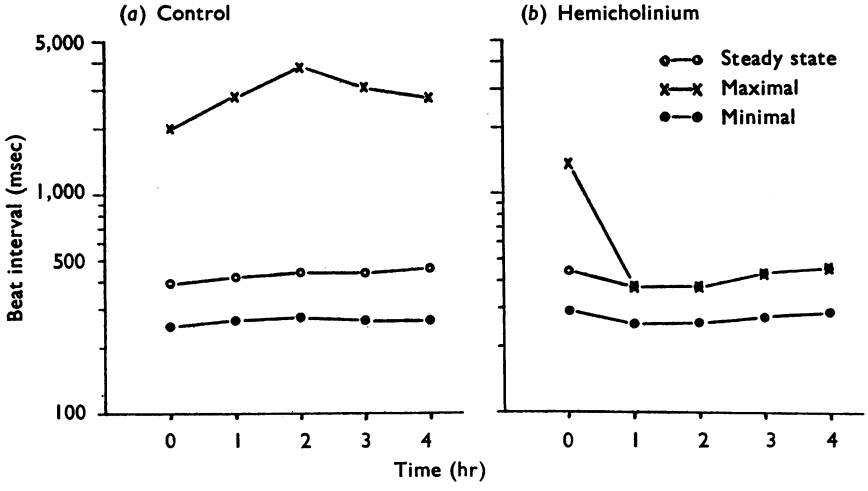

Fig. 3. Chronotropic responses of non-atropinized control and hemicholinium-treated preparations. Semilog plot of average steady state, maximal and minimal intervals against time. Steady state interval is the spontaneous beat interval just before intranodal nerve stimulation. Maximal and minimal intervals are the maximal and minimal spontaneous beat intervals, respectively, during or after intranodal nerve stimulation. Each point represents the mean of fifteen measurements from three preparations. (*a*), Control preparations, stimulated, but not treated with hemicholinium; (*b*), preparations treated with hemicholinium (5×10^{-4} g/ml.) beginning immediately after zero time. At 1 hr, there was complete block of the cholinergic electro-release response. There was no reduction in the adrenergic electro-release response.

the block by hemicholinium to occur. This last conclusion was substantiated by another kind of observation. Several preparations were stimulated in the described manner until complete block of the cholinergic electro-release response had occurred. Then the stimulating electrode was moved to another area of the sinoatrial node. The chronotropic response on stimulation of the new area was initially mixed, similar to that seen before hemicholinium. If the pattern of stimulation was continued in the new area progressive block of the cholinergic part of the response ensued.

No block of the adrenergic electro-release response was observed in any preparation treated with hemicholinium, even at concentrations of 5×10^{-4} g/ml. for as long as 4 hr (Fig. 3). Control experiments without hemicholinium were done to investigate the stability of the response to intranodal nerve stimulation. Results from non-atropinized and atropinized control experiments are illustrated in the left-hand panels of Figs. 3 and 4, respectively; the response to intranodal nerve stimulation was quite stable for the times and conditions used in these experiments. These results also serve as a basis for comparison with the results from hemicholinium-treated preparations.

In experiments of the type illustrated in Fig. 3, it is conceivable that the adrenergic electro-release response is maintained in the presence of hemicholinium because of an unmasking effect as the cholinergic response is blocked. To investigate this possibility, atropine (1×10^{-6}) was added to some preparations before and during perfusion with hemicholinium. As shown in Fig. 4, the adrenergic electro-release response was not depressed in the atropinized and hemicholinium-treated preparations over a period of 4 hr.

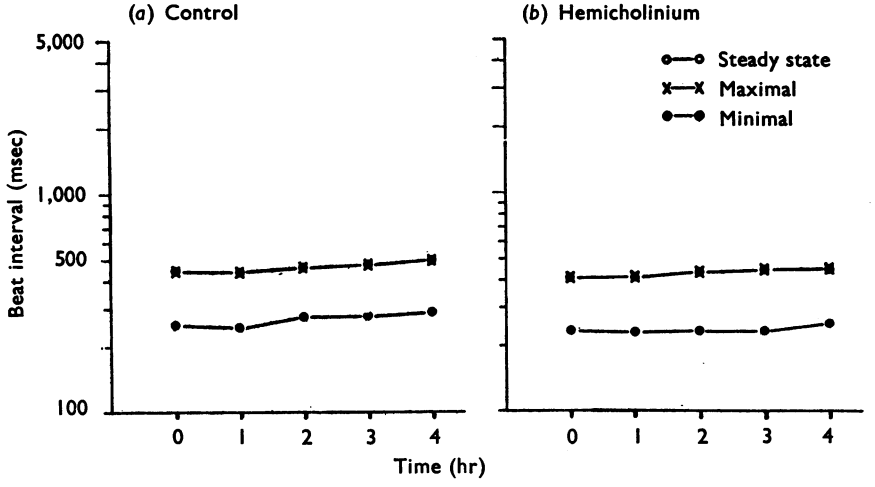

Fig. 4. Chronotropic responses of atropinized control and hemicholinium-treated preparations. Semilog plot of average steady state, maximal and minimal intervals against time. Steady state interval is the spontaneous beat interval just before intranodal nerve stimulation. Maximal and minimal intervals are the maximal and minimal spontaneous beat intervals, respectively, during or after intranodal nerve stimulation. Each point represents the mean of fifteen measurements from three preparations. (*a*), Control preparations, stimulated, but not treated with hemicholinium; (*b*), preparations treated with hemicholinium (5×10^{-4} g/ml.) beginning immediately after zero time. Since preparations were treated with atropine (1×10^{-6} g/ml.) throughout each experiment, the cholinergic electro-release response was completely blocked, and the maximal interval was equal to the steady state interval for each chronotropic response. The adrenergic electro-release response was not reduced in the hemicholinium-treated preparations.

To facilitate comparison of control and experimental adrenergic responses, the results illustrated in Figs. 3 and 4 were converted to percentage decreases in spontaneous beat intervals induced by nerve stimulation. The percentage decrease was calculated as the difference between the steady state interval and the minimal interval divided by the steady state interval and multiplied by 100. Table 1 summarizes those results pertinent to the test

TABLE 1

DECREASE IN SPONTANEOUS BEAT INTERVAL PRODUCED BY INTRANODAL NERVE STIMULATION OF ISOLATED SINOATRIAL NODE PREPARATIONS

Values represent the mean percentage decrease (with standard deviations) in spontaneous beat interval produced by a 10-sec burst of 50 shocks/sec intranodal nerve stimulation. Each value is the mean of fifteen measurements from three preparations. Atropinized preparations were treated with atropine (1×10^{-6} g/ml.) throughout, including the control period. Hemicholinium (5×10^{-4} g/ml.) was begun immediately after the control period in the experimental preparations. Control preparations were perfused and stimulated identically in the absence of hemicholinium

Time after hemicholinium (hr)	Decrease (%) in spontaneous beat interval			
	Non-atropinized		Atropinized	
	Control	Hemicholinium	Control	Hemicholinium
Before	36·1±0·7	34·2± 2·1	42·7±4·2	43·4±3·2
1	37·0±1·4	31·3±11·2	44·1±5·1	43·6±5·8
2	37·4±0·7	31·6±11·8	41·5±0·3	45·8±6·1
3	40·1±1·5	36·0± 9·6	41·9±1·1	46·5±6·0
4	42·2±0·9	38·3± 5·0	43·0±3·6	44·4±5·1

of the Burn & Rand (1959, 1962) hypothesis. Using Student's *t*-test none of the responses of the hemicholinium-treated preparations is significantly different from the appropriate control responses at the 5% level.

We have observed the action of hemicholinium in twenty-two different preparations from rabbits, and in each the results were qualitatively identical. Hemicholinium (in the presence of sufficient nerve stimulation) blocked the cholinergic electro-release response. Similar results were obtained in three cat sinoatrial node–right atrial preparations perfused with hemicholinium (5×10^{-4} g/ml.) for 4 hr.

DISCUSSION

It is well known that hemicholinium can block the synthesis of acetylcholine. MacIntosh (1961) reported that 10^{-3} M-hemicholinium (4.85×10^{-4} g/ml.) completely inhibited acetylcholine synthesis in minced brain tissue. Because of its ability to inhibit the synthesis of acetylcholine, and because of its reported inhibition of sympathetic nerve function, hemicholinium has been considered by some investigators to illustrate, more or less directly, the presence of a cholinergic mechanism in postganglionic sympathetic transmission (Burn & Rand, 1962). Implicit in such an interpretation is the assumption that, if hemicholinium inhibits acetylcholine synthesis, it should, in the presence of sufficient excitation to deplete preformed stores of the transmitter, inhibit any function subserved by a cholinergic mechanism. This would include any hypothetical cholinergic link in the release of noradrenaline from sympathetic nerve endings.

Results obtained in this study indicate that, under the conditions used, hemicholinium in a concentration of 5×10^{-5} g/ml. can completely block stimulated intranodal vagal fibres within approximately 1 hr. This block was shown to be unlike that produced by atropine and presumably depends on the inhibition of acetylcholine synthesis with subsequent depletion of preformed stores. The failure of block by hemicholinium to occur in the absence of nerve stimulation agrees with this interpretation. The observation that a cholinergic electro-release response could be obtained by moving the stimulating electrode to a new area of the sinoatrial node in preparations previously blocked by hemicholinium also agrees with this interpretation. In a previous publication we noted that the responses to bursts of direct subthreshold stimuli applied at two different points appeared to be additive (Vincenzi & West, 1963). We concluded that only some of the nervous elements in our preparations are excited by a given electrode pair. Thus, in this study, some vagal fibres in the sinoatrial node were not being excited during the initial stimulation and perfusion with hemicholinium. Those fibres which were stimulated (and which mediated the original negative chronotropic response) progressively lost their functional capabilities; as a result the cholinergic electro-release response was blocked. When the stimulating electrode was then moved to a new area of the sinoatrial node a new population of nerve fibres became available for stimulation. Since at least some of the vagal fibres in this new population were not stimulated during the perfusion with hemicholinium, their acetylcholine stores were not depleted. These fibres thus responded to stimulation with a release of acetylcholine and the cholinergic electro-release response was observed. Continued stimulation of the new population of nerve fibres in the presence of hemicholinium produced the expected block of the cholinergic electro-release response, but had no effect on the adrenergic electro-release response.

For two reasons, we believe the important site of action in the block by hemicholinium to be associated with the endings of postganglionic vagal fibres (rather than vagal ganglia). Lewartowski & Bielecki (1963), using the rabbit isolated vagus-atrial preparation treated with 1×10^{-5} g/ml. of hemicholinium, found that postganglionic parasympathetic function disappeared before, or simultaneously with, development of ganglionic blockade. Furthermore, since hexamethonium does not alter the observed response to intranodal nerve stimulation (Lewartowski, 1963), preganglionic parasympathetic excitation, if it occurs, is incidental and unimportant in the resultant negative chronotropic response. Lewartowski has advocated the isolated vagus-atrial preparation as a test system for studying vagolytic drugs. When prepared so as to compare responses to intranodal nerve fibre stimulation (postganglionic) with responses to vagal trunk stimulation (preganglionic), information on the site of action of such drugs can be obtained (Lewartowski, 1963).

At a concentration of 5×10^{-4} g/ml., or ten-times that shown to block completely the cholinergic electro-release response, hemicholinium did not significantly reduce the adrenergic electro-release response. Thus, hemicholinium was effective in blocking classically cholinergic vagal endings, but was not effective, even in higher concentrations, in blocking classically adrenergic accelerator nerve endings. This result differs from that reported by Chang & Rand (1960), who showed that hemicholinium (5×10^{-4} g/ml.) blocked the positive chronotropic response to nerve stimulation in cat isolated sympathetically-innervated atria. We treated three cat preparations with hemicholinium (5×10^{-4} g/ml.) for 4 hr and found no block of the positive chronotropic response to intranodal nerve stimulation. Thus a species difference cannot account for the differing results. In the Chang & Rand experiments stimuli were applied to the isolated stellate ganglion and perhaps there was, to some extent, preganglionic excitation. Since hemicholinium would be expected to block ganglionic transmission, this could be a factor in their results.

Since block by hemicholinium apparently depends on transmitter depletion, perhaps the most meaningful comparison of our results and those of Chang & Rand can be made by considering the total number of stimuli delivered during hemicholinium administration. Chang & Rand indicated complete block of the sympathetically induced positive chronotropic response in cat atria in 160 min with 5×10^{-4} g/ml. of hemicholinium. With their mode of stimulation, this time would include 19,200 stimuli. On the other hand, using the same concentration of hemicholinium in the rabbit or cat sinoatrial node, we delivered 36,000 stimuli in 240 min and found no decrease in the positive chronotropic response to intranodal nerve stimulation. The results demonstrate that sympathetic nerves can function in the presence of a high concentration of hemicholinium with rigorous stimulus parameters over a long period of time. The results thus provide no evidence to support the hypothesis of a cholinergic link in the release of noradrenaline in the sinoatrial node.

SUMMARY

1. Rabbit and cat isolated sinoatrial node–right atrial preparations were subjected to selective stimulation of intranodal autonomic nerve fibres. Nerve stimulation (10 V, 50 shocks/sec, 0.05 msec duration) was applied to the sinoatrial node in 10-sec bursts every 200 sec. This procedure elicited a biphasic chronotropic response which was stable for the experimental times employed.

2. Changes in spontaneous beat interval were used as a measure of the release of acetylcholine and noradrenaline induced by intranodal nerve stimulation.

3. Hemicholinium (5×10^{-5} g/ml.) completely blocked the cholinergic response to intranodal nerve stimulation in about 1 hr. On the other hand, at concentrations as high as 5×10^{-4} g/ml., hemicholinium did not reduce the adrenergic response to intranodal nerve stimulation within 4 hr.

4. Hemicholinium had no effect on the adrenergic response in atropinized (1×10^{-6} g/ml.) preparations.

5. The results do not support the hypothesis of a cholinergic link in the release of noradrenaline from accelerator nerve endings in the sinoatrial node.

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