Brit. J. Pharmacol. (1947), 2, 131.

THE ACTION OF ACETYLCHOLINE, ADRENALINE AND OTHER SUBSTANCES ON THE REFRACTORY PERIOD OF THE RABBIT AURICLE

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(Received January 17, 1947)

In discussing the action of quinidine, veratrine, and strophanthin on the refractory period of cardiac muscle, Lewis and Drury (1926) pointed out that the effect on the absolute refractory period differed from that on the effective refractory period. The absolute refractory period is the length of time before the muscle will again respond to a stimulus; the effective refractory period is the time before the muscle will again transmit a propagated wave to any distance, and for most purposes it is this effective refractory period which is important. Thus Drury and Love (1926) found that quinidine actually shortened the absolute refractory period of the tortoise heart, but lengthened the effective refractory period; it is, of course, this action on the effective refractory period which makes quinidine of value to restore the normal rhythm in auricular fibrillation.

A method of measuring the effective refractory period in the auricular muscle of the rabbit heart has recently been described by Dawes (1946). Using this method to test different substances for their power to act as substitutes for quinidine and to prolong the refractory period of the auricular muscle, Dawes found that substances with several different pharmacological actions were effective. Thus the local anaesthetics amethocaine, cocaine, and procaine prolonged the refractory period; similarly, substances called spasmolytics, such as syntropan, trasentin, pethidine, and papaverine, also prolonged the refractory period. Dawes pointed out that procaine was not only a local anaesthetic, but had a spasmolytic action, shown by its power to reduce the stimulant action of acetylcholine on the rabbit intestine, and further that procaine diminished the action of acetylcholine in inhibiting the amplitude of contraction of the rabbit auricles beating spontaneously. Dawes states that it is remarkable that quinine, quinidine, and procaine antagonize the action of acetylcholine on many different types of tissue. He quotes Harvey (1939a, b), who showed that quinine and procaine reduced the action of acetylcholine on denervated skeletal muscle; he quotes Macgregor (1939), who showed that procaine reduced the pressor response

to acetylcholine in the atropinized cat, and also Stavraky (1932), who showed that quinine reduced the secretory action of acetylcholine on the salivary gland. It appears that many of the substances which prolong the refractory period have the general property of antagonizing the action of acetylcholine.

The question therefore arose what the action of acetylcholine itself on the refractory period of the auricular muscle might be, and the work described here concerns this action. Observations have recently been made by Wedd and Blair (1945) on the action of acetylcholine and adrenaline on the turtle ventricle. They studied the changes in the Q–T interval of the electrocardiogram. With concentrations of acetylcholine equal to 1 in 100,000 or higher, they observed slight shortening of the Q–T interval. In an earlier paper, Blair, Wedd and Young (1941) discussed the relation of the Q–T interval to other events in the cardiac cycle, and concluded that the Q–T interval coincided with the absolute refractory period. Wedd and Blair therefore consider that since acetylcholine and also carbaminoylcholine reduce the Q–T interval, they also reduce the absolute refractory period. In the paper by Drury and Love (1926) in which they distinguished between the refractory period as ordinarily measured and the absolute refractory period, it was shown that whereas quinidine lengthened the refractory period as ordinarily measured, it diminished the absolute refractory period. It is therefore clear that no conclusion can be drawn about the effect of a drug upon the effective refractory period from its effect upon the absolute refractory period.

Dawes (1946) has already stated that adrenaline diminished the refractory period when it was tested on the isolated rabbit auricle. He did not, however, publish any figures relating the dose to the effect.

Experimental Results

Acetylcholine.—The preparation of the rabbit auricle was the same as described by Dawes (1946).

To test the effect of acetylcholine, 12 observations have been made in 4 preparations. As an example of the effect, before the addition of acetylcholine the auricle was able to follow stimulation at the rate of 260 per min., but not at the rate of 274 per min.; each rate was applied twice with the same result. After 10 μg. acetylcholine had been added, the preparation followed stimulation first at 274 per min., then at 292 per min., but failed to follow at 314 per min.; it then followed again at the rate 292 per min. Thus a concentration of 1 in 10 million shortened the refractory period by 12 per cent. Larger doses produced a greater effect, and the addition of 50 μg. enabled the auricle to follow a rate 40 per cent greater than the previous maximal rate. The different observations are given in Table I. In all the diminution of the effective refractory period is clearly seen. The relation between the percentage increase in the maximal rate at which the auricles would follow the imposed rhythm was found to be in linear

TABLE I

EFFECT OF ACETYLCHOLINE IN SHORTENING THE REFRACTORY PERIOD

Experiment No.	Dose μg.	Rate per min. at which auricle followed		Increase %
		before acetylcholine	in presence of acetylcholine	
1	2.5	292	338	15.7
	5.0	292	350	19.8
	10.0	292	363	24.0
2	50.0	284	400	40.9
	50.0	284	362	27.4
3	5.0	314	338	7.5
	10.0	314	362	15.2
	20.0	314	362	15.2
	40.0	314	388	24.0
	40.0	314	388	24.0
	80.0	314	414	32.0
4	10.0	260	292	12.3

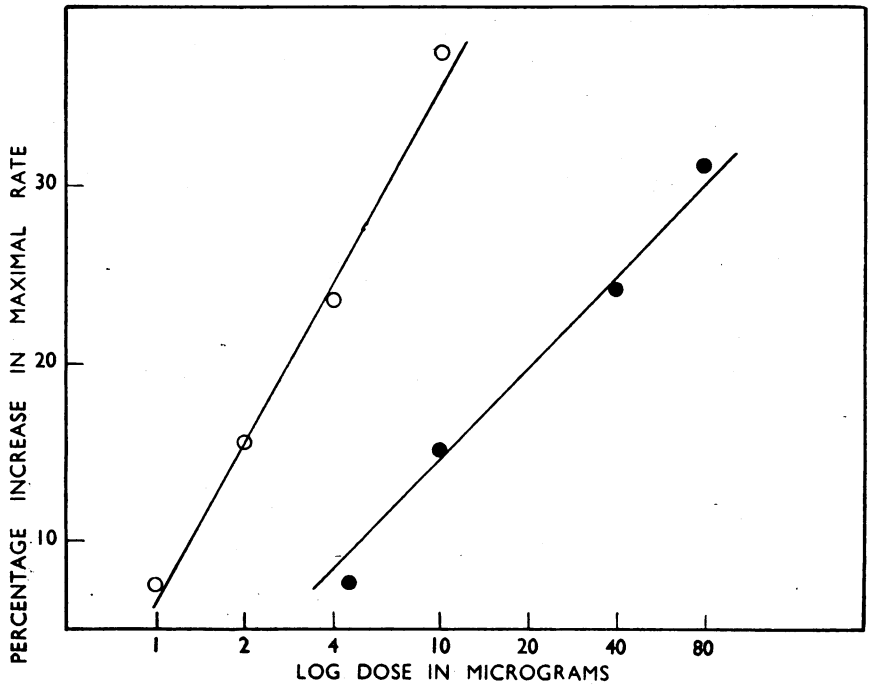

FIG. 1.—Ordinates; per cent increase in maximum rate at which the auricle responded to electrical stimuli. Abscissae: doses (bath of 100 ml.) on a logarithmic scale. The black circles show the effect of acetylcholine; the white circles show the effect of carbaminoylcholine.

proportion to the logarithm of the concentration of acetylcholine in one experiment. This is shown in Fig. 1. The doses of acetylcholine used diminished the amplitude and the frequency of the spontaneous contractions of the auricles, though even the largest dose, which corresponded to a concentration of 1 in 1,250,000, did not cause complete arrest.

Carbaminoylcholine.—Wedd and Blair (1945) found that carbaminoylcholine diminished the Q–T interval of the turtle ventricle, and I have found that, like

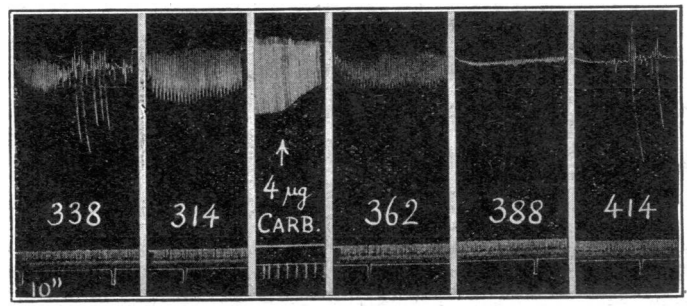

FIG. 2.—Isolated rabbit auricles driven electrically. On the left the auricles fail to follow 338 stimuli per min., but follow 314 per min. regularly. At the arrow 4 μg. carbachol was added to the bath. This diminished the amplitude. The auricles then followed 362 and 388 stimuli per min., though at this high rate the amplitude was very small. At 414 per min. the auricles did not follow the stimuli.

acetylcholine, carbaminoylcholine increases the maximum rate at which the auricles will follow an applied stimulus. The spontaneous contractions of the auricles were arrested, but the responses to the electrical stimulation were more

TABLE II

EFFECT OF CARBAMINOYLCHOLINE IN SHORTENING THE REFRACTORY PERIOD

Experiment No.	Dose μg.	Rate per min. at which auricle followed		Increase %
		before carbaminoylcholine	in presence of carbaminoylcholine	
2	50	292	430	47.2
5	25	252	304	20.6
	50	244	304	24.5
6	1	314	338	7.5
	2	314	362	15.5
	4	314	388	22.5
	8	314	430	37.7
	10	314	430	37.7
7	1	292	338	15.7
	2	292	338	15.7
	4	292	362	23.9
	8	292	388	32.7
	16	292	412	41.0

vigorous than usual. A concentration of 1 in 100 millions increased the maximal rate by 5.5 per cent, and concentrations in the neighbourhood of 1 in 10 millions increased the maximal rate by about 40 per cent. The observations are given in Table II, and the relation between the logarithm of the dose and the effect in one experiment is shown in Fig. 1. An illustration of the effect on the auricles is given in Fig. 2.

Adrenaline.—To determine the effect of adrenaline on the maximal rate of stimulation was a more difficult matter. As already mentioned, Dawes found that adrenaline enabled the auricle to follow a higher rate than before and therefore shortened the refractory period. In most experiments I have confirmed this observation, but in others adrenaline had the opposite effect, at least at certain points (see Table III). Out of 28 observations, adrenaline enabled the

TABLE III

IMMEDIATE EFFECT OF ADRENALINE ON REFRACTORY PERIOD

Experiment No.	Dose μg.	Rate at which auricle followed			Immediate change %
		before adrenaline	in presence of adrenaline	after one washing	
8	10	274	292	260	6.2
	20	274	314	284	14.6
	40	274	378	292	19.0
	80	292	378	—	29.4
9	10	362	338	292	–6.6
	10	292	274	274	–6.1
	20	274	292	274	6.2
	40	274	314	314	14 6
	80	274	292	274	6.2
	160	274	314	274	14.6
	300	274	292	274	6.2
	600	274	274	—	0
10	20	260	274	260	5.3
	40	260	274	260	5.3
11	10	314	338	274	7.6
	10	314	338	274	7.6
	20	314	362	338	15.3
	20	314	338	292	7.6
	40	274	314	292	14.6
	80	292	314	292	7.5
12	10	274	292	244	6.2
	10	260	292	260	12.3
	20	274	338	260	23.3
	40	274	314	234	14.6
13	10	274	274	260	0
	20	274	274	244	0
	40	274	292	274	6.5
	40	274	292	260	6.5

auricle to follow at a higher rate in 23, but in the remaining 5 observations the rate was unaffected or reduced. As examples of reduction, the addition of 10 μg. adrenaline to the bath of 100 ml. reduced the rate from 362 to 338 per min. and, in a second trial, from 292 to 274 per min. The changes in the maximum rate

TABLE IV

THE NUMBER OF TIMES A GIVEN CHANGE WAS OBSERVED

Dose of adrenaline μg. in 100 ml.	Maximum rate			
	increased more than 10%	increased less than 10%	not changed	decreased
10	1	4	1	2
20	3	3	1	
40	4	3		
80	1	2		

caused by adrenaline are shown in Table IV, in which it is seen that as the concentration of adrenaline was increased more observations were made in which the maximum rate was increased. Thus when 10 μg. was added to the bath, an

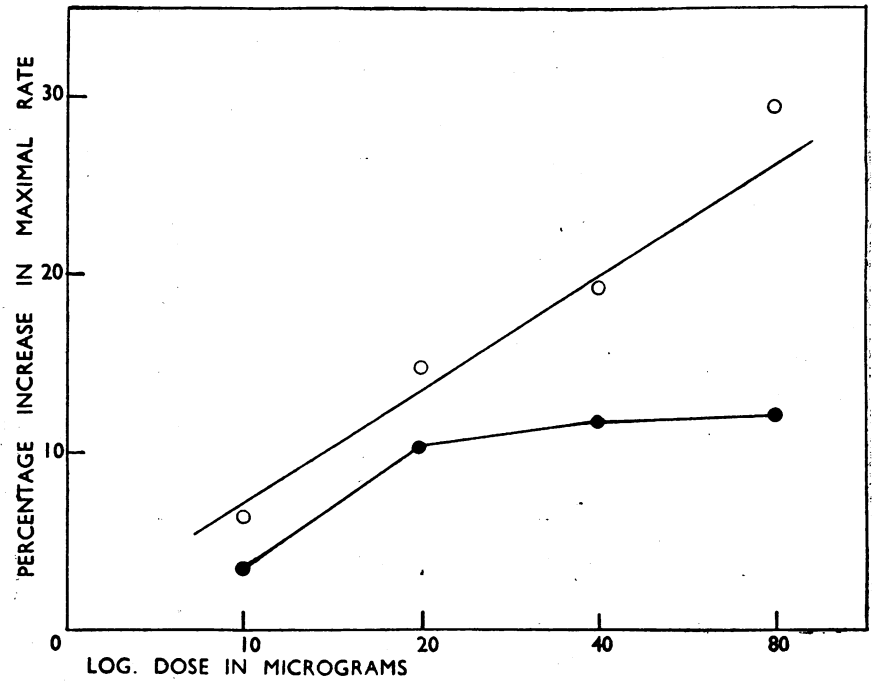

FIG. 3.—Ordinates and abscissae as in Fig. 1. The white circles show the results obtained with different concentrations of adrenaline in one experiment. The black circles are the mean results of all the observations made with adrenaline.

increase greater than 10 per cent was observed in 1 out of 8 trials, whereas when 40 μg. was added such an increase was observed in 4 out of 7 trials. The mean of all observations is shown in Fig. 3, in which the results of one exceptional experiment are also shown where the percentage increase in maximum rate was in linear relation to the logarithm of the dose. In some experiments the higher doses of adrenaline produced a smaller increase in maximal rate than lower doses.

A further curious observation was made that when adrenaline was added to the bath and then washed out, the maximum rate fell below the initial rate; thus the refractory period was first shortened by adding adrenaline, and then lengthened by washing it out. After further washing the rate returned by stages

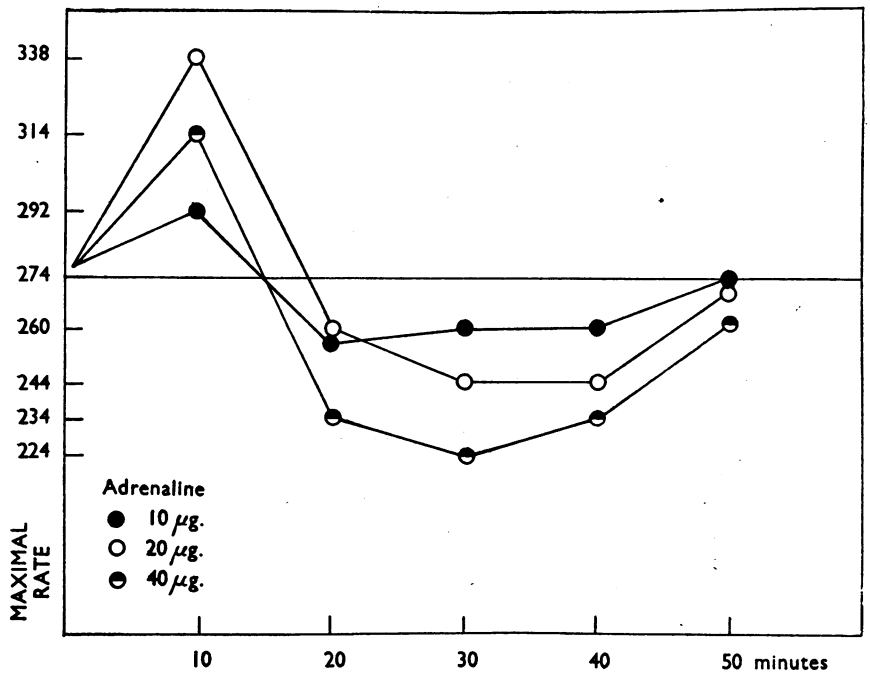

FIG. 4.—Ordinates: maximum rate per min. at which the auricles responded to electrical stimuli. Abscissae: time in minutes. The adrenaline added to the bath was in contact with the auricles only during the first 10 min.; after washing out at 10 min. intervals, the maximum rate fell below the initial rate, and then gradually returned.

to the initial rate. Examples of this effect are given in Fig. 4. This succession of changes caused by adrenaline was observed 9 times in 4 different experiments.

Nicotine.—An action of nicotine (used as acid tartrate) on the isolated rabbit auricle was exerted when large doses were given; these produced an increase in amplitude as shown in Fig. 5; no inhibitory effect was seen. Doses less than 0.8 mg. (added to the bath of 100 ml.) had no effect on the refractory period,

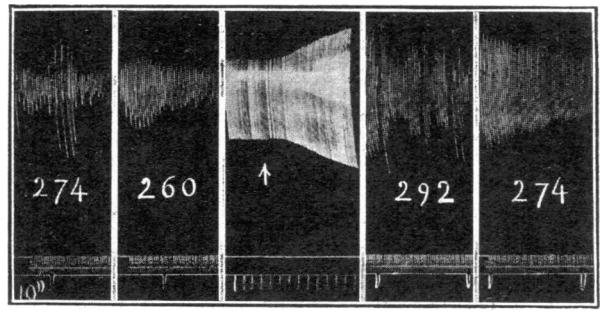

FIG. 5.—Similar to Fig. 2. This record is shown to demonstrate the effect of 1.6 mg. nicotine acid tartrate added at the arrow. Note the increase in amplitude similar to that produced by adrenaline; no inhibition is seen. After the addition of nicotine the auricles followed 274 stimuli per min., whereas before the addition the maximum rate was 260 per min.

but doses equal to this, or greater, usually increased the rate at which the auricles followed the applied stimulus, though the effect was small. In several observations, however, this was the initial effect only, and while the nicotine remained in the bath, the rate fell below the starting rate. Among the results given in Table V, there are examples of this double effect in each experiment. Thus in Exp. 4, the addition of 3.0 mg. caused an initial increase from 274 to 292 per

TABLE V

IMMEDIATE EFFECT OF NICOTINE ON THE REFRACTORY PERIOD

Experiment No.	Dose mg.	Rate at which auricle followed			Change (%)
		before nicotine	in presence of nicotine	after one washing	
4	0.4	274	274	274	0
	0.8	274	260	244	− 5.1
	1.6	260	274	274	+ 5.3
	3.0	274	292		+ 6.5
			224	234	−18.2
	1.6	274	244	260	−10.6
13	0.08	274	274	266	0
	0.2	266	266	266	0
	0.8	266	274		+ 3.0
			238	266	−10.5
	1.6	266	284		+ 6.7
			238	266	−10.5
	3.0	266	304		+14.2
			252		− 5.2
14	0.8	314	338	292	+ 7.6
	1.6	292	326		+11.6
			292	314	− 7.5
	3.0	292	314		+ 7.5
			244	314	−16.4
	1.6	314	338	292	+ 7.6

min., followed by a decrease to 224 per min. before washing out. The two phases of the nicotine action appeared to correspond to a stimulant stage and a paralysing stage. Thus, after testing the effect of 3.0 mg., a retrial of the effect of 1.6 mg. produced a fall in the maximal rate, whereas earlier it produced a rise.

Prostigmine.—The effect of prostigmine was tested in doses of 1 mg. and 2 mg. These amounts produced either no effect or slight increases of 5 or 6 per cent in the maximal rate ; they greatly augmented the effects of acetylcholine and of nicotine, as shown in Table VI. Whereas 200 μg. of acetylcholine alone increased the rate by 39 per cent, in the presence of 1.0 mg. prostigmine 10 μg. of acetylcholine increased the maximum rate by 57 per cent.

TABLE VI

EFFECTS IN THE PRESENCE OF PROSTIGMINE

Substance	Dose μg.	Rate at which auricle followed			Change %	
		before drug	in presence of drug	after one washing	(a)	(a) to (b)
Exp. 15						
acetylcholine	200	260	362 (a)	292	39	
prostigmine	1,000	260	274 (a)		5	
acetylcholine	10		430 (b)	260		57
prostigmine	2,000	260	260 (a)		0	
acetylcholine	20		388 (b)	292		49
nicotine	1,600	260	388 (a)	260	49	
acetylcholine	10	260	388 (a)	244	49	
Exp. 16						
prostigmine	1,000	274	292 (a)		6	
acetylcholine	100		388 (b)	314		33
prostigmine	2,000	292	292 (a)		0	
acetylcholine	200		430 (b)	274		47
prostigmine	2,000	274	292 (a)		6	
nicotine	1,500		362 (b)	274		24
prostigmine	2,000	274	292 (a)		6	
nicotine	3,000		362 (b)	274		24
prostigmine	2,000	274	292 (a)		6	
nicotine	1,500		292 (b)	274		0
nicotine	1,500	274	274 (a)		0	

The increases in maximal rate produced by nicotine and recorded in Table V were often small ; in the presence of prostigmine, however, they were very large ; thus 1.6 mg. nicotine added to the auricle when some prostigmine effect remained (after washing out 2 mg. prostigmine), increased the maximum rate by 49 per cent.

An attempt was made to see if large doses of acetylcholine, added in the presence of prostigmine, would depress the maximum rate. No such depression was observed, as shown in the second part of Table VI, though the repetition of large doses of nicotine led to disappearance of their action.

Eserine.—Eserine acted similarly to prostigmine. Doses of 200 μg. were themselves without effect, but in their presence the action of acetylcholine was increased ; thus 1, 2, and 4 μg. acetylcholine produced increases of 14.5, 20, and 39 per cent in the maximal rate. Eserine likewise increased the action of nicotine ; in the presence of 200 μg. eserine, nicotine in a dose of 0.1 mg. increased the maximum rate by 14.5 per cent, but larger doses of 0.2 mg. and 0.4 mg. nicotine tested afterwards had less action, the increase being 6.5 per cent for each dose.

Atropine.—When atropine in a dose of 1 mg. was added to a fresh preparation there was, in each of three experiments, a slight diminution in the maximum rate. After this addition, acetylcholine failed to cause the usual increase. In some experiments it appeared that after atropine, the action of adrenaline disappeared, but this was not always so, and in view of the variation in the response to adrenaline, it was not possible to conclude that atropine affected its action ; nor did atropine modify the action of nicotine.

Discussion

The results obtained in this work are in line with the points brought out by Dawes concerning the substances which resemble quinidine in its action on the electrically driven auricle. Dawes found that local anaesthetics and spasmolytics have a quinidine-like action and that they share the property of antagonizing the action of acetylcholine in stimulating the isolated intestine of the rabbit and in inhibiting the spontaneous contractions of the isolated rabbit auricles. He pointed out that on skeletal muscle also the action of a local anaesthetic like procaine resembles that of quinine. From Dawes's observations the conclusion can therefore be drawn that substances which prolong the refractory period of cardiac muscle are, in general, antagonists of acetylcholine. The inference from this conclusion is that acetylcholine, and substances with a similar action, should shorten the refractory period. The observations described in this paper show that acetylcholine has this action and that carbaminoylcholine has the same action. The effect of acetylcholine is increased in the presence of eserine or prostigmine, and it is abolished in the presence of atropine. The action of adrenaline is similar in direction to that of acetylcholine, but as the dose of adrenaline is increased there is little increase in effect. With acetylcholine there is a linear relation between log dose and the increase in the maximum rate at which auricles can be driven over a considerable range. With adrenaline this is not so ; with increasing doses of adrenaline the increase in maximum rate is much less, and soon reaches a point beyond which no further increase is observed.

Since the diverse substances which have a quinidine-like action on the auricles have the common property of antagonizing the action of acetylcholine, the suggestion arises that the transmission of the impulse in cardiac muscle may be effected by a mechanism in which acetylcholine is a key substance and in which

the rate of transmission is governed by the rate of formation of acetylcholine. Abdon and Hammarskjöld (1944) have demonstrated that rabbit hearts, among other tissues, contain a precursor from which acetylcholine can be liberated, so that such a mechanism is a possibility. The application of acetylcholine to the isolated auricle might then be supposed to facilitate the working of the mechanism by providing acetylcholine ready made. The action of adrenaline could be regarded as an action in which it potentiated the effect of acetylcholine, the extent of the potentiation being limited. Other examples of the potentiation of acetylcholine by adrenaline have been described by Bülbring and Burn (for references, see Burn, 1945). The action of acetylcholine in shortening the refractory period is abolished by atropine, and if acetylcholine is concerned in the transmission of the normal process of excitation, it might be expected that atropine would be a potent substance in prolonging the refractory period. Several examples, however, are known where a normal process mediated by acetylcholine is not appreciably affected by atropine, although atropine abolishes the action of acetylcholine when externally applied. When, however, such a normal process is exaggerated, then atropine removes the exaggeration. Novoa Santos in his textbook of general pathology (1934) describes how auricular fibrillation in a proportion of patients is arrested by atropine ; if it is supposed that in these cases the fibrillation is due to excessive formation of acetylcholine, and that atropine antagonizes the action of this excess, we can then understand how atropine acts. In a similar fashion, we can understand how atropine removes the tremors of paralysis agitans without affecting ordinary voluntary movements. Ordinary movements appear to require the mediation of acetylcholine at the synapses ; spinal reflexes are not, however, modified by atropine. The effect of accumulations of acetylcholine in the spinal cord, whether inhibiting or stimulating, is, however, abolished by atropine (Bülbring and Burn, 1941).

SUMMARY

1. Observations have been made on the isolated auricles of the rabbit driven electrically. Acetylcholine and carbaminoylcholine increase the maximum rate at which the auricles will follow the stimulation applied.

2. The relation between the percentage increase in maximum rate and the logarithm of the dose is linear for both these substances.

3. The effect of acetylcholine is increased by eserine or prostigmine and abolished by atropine.

4. Adrenaline has some effect in increasing the maximum rate of stimulation, but this effect increases only slightly with increase of dose.

5. Nicotine has an initial acetylcholine-like action in large doses, which is followed by the opposite effect, probably owing to partial paralysis.

6. The mechanism of these changes is discussed.

I wish to express my thanks to Prof. J. H. Burn for his guidance and advice throughout this work.

This work was carried out during the tenure of a grant from the Spanish Council for Scientific Research.

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