Brit. J. Pharmacol. (1964), **22**, 176–182.

THE SYMPATHETIC MECHANISM IN THE ISOLATED PULMONARY ARTERY OF THE RABBIT

BY

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(*Received October* 10, 1963)

The nature of postganglionic sympathetic nervous transmission to vascular muscle *in vitro* was studied using the recurrent cardiac nerve-pulmonary artery preparation of the rabbit. Experiments, similar to those which in other tissues have provided evidence to support a role for acetylcholine at the sympathetic postganglionic nerve-effector cell junction, were carried out. The contractile response of the isolated artery to acetylcholine was blocked completely by atropine. High concentrations of acetylcholine and of hemicholinium had no effect on the contractile response to sympathetic nerve stimulation. Physostigmine, atropine and hemicholinium were without influence on the relationship between nerve stimulus frequency and response. Yohimbine, bretylium and reserpine blocked completely the response to nerve stimulation but did not affect that to applied acetylcholine. These results support the view that transmission in this preparation at the sympathetic postganglionic nerve-effector cell junction is mediated by an adrenaline-like transmitter and provide no evidence for the view that acetylcholne is involved at this site.

In 1959, Burn & Rand postulated that an impulse passing along a postganglionic sympathetic nerve fibre released acetylcholine at its termination, which in turn liberated the sympathetic transmitter. Subsequently, considerable indirect evidence has been marshalled in support of this hypothesis (Burn, 1963). On the basis of the distribution of acetylcholinesterase in adrenergic nerves, Koelle (1962) also has suggested that acetylcholine may play a part in transmission at some sympathetic terminals. On the other hand, several alternative interpretations of the observations on which the hypothesis of Burn & Rand is based have been put forward (see discussion after Burn, 1960 ; Gillespie & MacKenna, 1961 ; Ferry, 1963). Recent studies on the nictitating membrane (Gardiner, Hellman & Thompson, 1962 ; Nystrom, 1962 ; Trendelenburg, 1962), vas deferens (Bentley, 1962 ; Bentley & Sabine, 1963 ; Jacobowitz & Koelle, 1963), spleen (Blakeley, Brown & Ferry, 1963) and blood vessels (Adams & Bay, 1963 ; Whelan & Skinner, 1963) appear to question the general applicability of the hypothesis and prompt further investigation.

Using the isolated sympathetic nerve-pulmonary artery preparation of the rabbit (Bevan, 1962), we have performed experiments, analogous to those of other workers, which appear to provide support for a role of acetylcholine at the sympathetic postganglionic terminal. Our experiments provide no support for the application of the hypothesis of Burn & Rand to this preparation.

METHODS

The method has been described by Bevan (1962). The distal 5 mm of the rabbit pulmonary artery with the right recurrent cardiac nerve was isolated and mounted in an organ-bath (50 ml. volume) containing Krebs solution bubbled with a mixture of 95% oxygen and 5% carbon dioxide and maintained at 38° C. Isometric contractions of the circular muscle were recorded using a sensitive strain gauge on a strip chart recorder following indirect and direct electrical stimulation before and after the addition of drugs to the bath fluid. The nerve electrodes were always placed at 3 mm or more from the vessel wall.

The relationship between the frequency of indirect stimulation and the contractile response was determined by measuring the maximal tension developed in responses to trains of 200 stimuli of supramaximal voltage and 2 msec duration, delivered at test frequencies of 2, 5, 10, 20, 40, 50, 60, 80 and 100 shocks/sec in random sequence. The responses to two test frequencies were bracketed by those to a standard frequency of 25 shocks/sec. These responses were elicited at 4 min intervals and expressed as percentages of the mean tension of the two adjacent responses to the standard stimulus frequency. After this sequence had been carried out, the tissue was exposed to the drug being studied for 1 hr during which period an indirect stimulation (200 stimuli at 25 shocks/sec) was applied every 4 min. Then the frequency/response measurements were repeated within the following hour. Responses to single direct electrical stimuli were elicited before and after each determination of the frequency/response relationship.

Drug doses are given as g of salt/ml. of bath solution. Acetylcholine chloride, physostigmine salicylate, atropine sulphate, hemicholinium (HC-3) dibromide, choline chloride, bretylium tosylate and yohimbine hydrochloride were used.

RESULTS

Acetylcholine

Acetylcholine contracted the rabbit resting isolated pulmonary artery preparation. The threshold concentration was approximately 1×10^{-8} but was less if the preparation had been exposed to physostigmine (1.5×10^{-5} in the bath fluid). This response to acetylcholine (Fig. 1, Ach) was completely blocked after the tissue had been exposed to a relatively low concentration of atropine (2×10^{-7}) for 30 min. A very high concentration of acetylcholine (1×10^{-3}) had no action on the response of the artery to sympathetic nerve stimulation (Fig. 1).

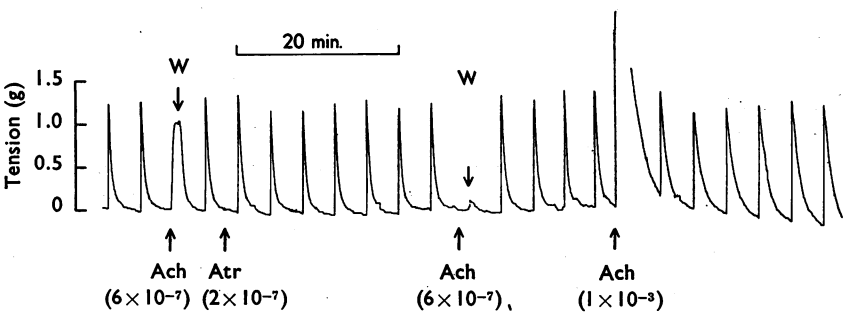

Fig. 1. Contractile responses of the rabbit isolated pulmonary artery preparation to sympathetic nerve stimulation and to acetylcholine. 200 stimuli at a frequency of 25 shocks/sec were delivered to the nerve at 4 min intervals, except during the cycles when acetylcholine (Ach) was applied. Washings (W) caused small artifacts. After addition of acetylcholine (1×10^{-3}), the baseline was adjusted so as to be below the original, as the tissue did not completely relax after addition of this concentration of acetylcholine. Atropine (2×10^{-7}) was added at Atr.

Physostigmine and atropine

Physostigmine (up to 1.5×10^{-4}) and atropine (up to 1×10^{-6}) did not influence the height of contraction following indirect stimulation at a frequency of 25 shocks/sec. Nor was there any transient response to these drugs (Table 1). Due to variation in the amplitude of responses, comparisons were made between the mean sizes of grouped responses taken within hourly periods, rather than between individual ones at a given time. The inhibition of response seen with the higher concentration of atropine (1×10^{-5}) was probably not due to a cholinergic blocking action, for the response to direct electrical stimulation was also reduced by approximately 75% after 2 hr of exposure with reference to the control experiments.

TABLE 1

EFFECT OF DRUGS ON THE ISOMETRIC RESPONSE OF THE RABBIT ISOLATED PULMONARY ARTERY PREPARATION TO SYMPATHETIC NERVE STIMULATION

Responses of the isolated artery to nerve stimulation (trains of 200 stimuli, duration 2 msec, frequency 25 shocks/sec) were elicited every 4 min and those to single direct muscle stimuli every 1 hr. After a preliminary control period of 1 hr, drugs were added to the bath fluid and their effects followed for a further 2 hr. The mean isometric responses during each of these 2 hr are compared with the mean response before treatment with drugs. *P* refers to the significance of the difference between the means of the treated and control values determined by the " t " test

		Response (% of mean control value) during treatment for			
		1st hr		2nd hr	
Treatment	No. of expts	Mean±s.e.	*P*	Mean±s.e.	*P*
Control	7	75·0±4·6	—	61·4±5·3	—
Physostigmine ($1{\cdot}5 \times 10^{-5}$)	5	81·2±4·9	>0·3	64·6±10·8	>0·7
Physostigmine ($1{\cdot}5 \times 10^{-4}$)	3	67·2±4·4	>0·3	58·0±4·7	>0·7
Atropine (1×10^{-6})	3	67·8±3·4	>0·3	66·5±4·5	>0·5
Atropine (1×10^{-5})	2	16·4, 25·6	—	17·3, 17·6	—
Hemicholinium (1×10^{-4})	3	67·5±2·0	>0·3	48·3±5·1	0·2>*P*>0·1
Hemicholinium (3×10^{-4})	3	61·8±6·4	0·2>*P*>0·1	43·8±8·0	0·1>*P*>0·05
Hemicholinium (1×10^{-3})	2	60·0, 44·7	—	33·7, 35·3	—

In some experiments, after the relationship between stimulus frequency and response had been established, physostigmine (1.5×10^{-5} and 1.5×10^{-4}) or atropine (1×10^{-6}) was added to the tissue bath for 1 hr. The shape of the frequency/response curve following either drug was not different from that in the control (Fig. 2).

Hemicholinium

Hemicholinium, which inhibits acetylcholine synthesis in some nervous tissues and is antagonized by choline (MacIntosh, Birks & Sastry, 1956), in concentrations as high as 1×10^{-4} affected neither the frequency/response relationship nor the absolute size of response (Table 1). The depression of the response by the high dose of hemicholinium (1×10^{-3}) was associated with a reduction in response to direct stimulation (65% in 2 hr), as in the vas deferens (Bentley & Sabine, 1963), and was not reversed by choline (5×10^{-4}).

Bretylium and yohimbine

Bretylium (1×10^{-6}) and yohimbine (1×10^{-5}) completely blocked the effect of indirect stimulation in about 1 hr, but had no influence on the response to acetyl-

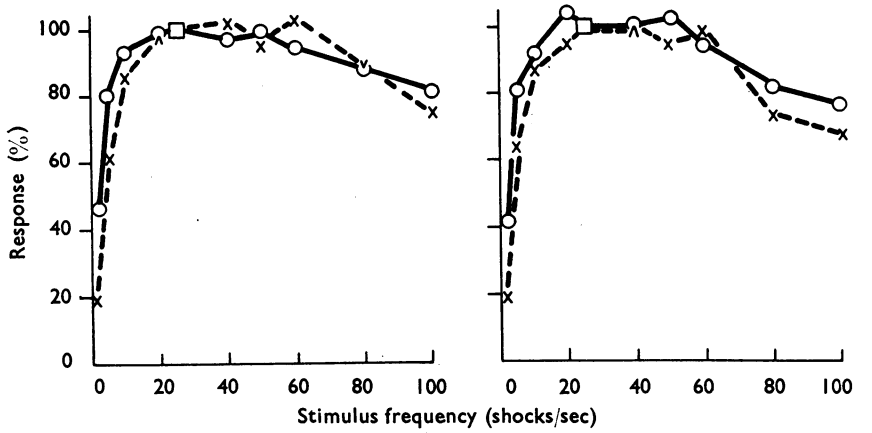

Fig. 2. Isometric response of the rabbit isolated pulmonary artery preparation (ordinate) to 200 stimuli applied to the nerve at various frequencies (abscissa), expressed as a percentage of the response at 25 shocks/sec. The solid lines indicate the initial measurements and broken lines those made 1 hr later. In (*b*), physostigmine ($1{\cdot}5\times10^{-5}$) was added immediately following the initial measurements. Each point represents the average of eight observations in (*a*) and of six in (*b*).

choline. If acetylcholine was released at the postganglionic sympathetic nerve terminal, a cholinergic response might be expected from nerve stimulation in the presence of these antiadrenaline agents. With complete block to nerve stimulation no response was seen despite treatment of the preparation with physostigmine which presumably should have enhanced any response to acetylcholine (Fig. 3).

Treatment of the rabbit with reserpine (5 mg/kg, intraperitoneally) 48 hr before and again 24 hr (5 mg/kg, intravenously) before dissection or soaking the

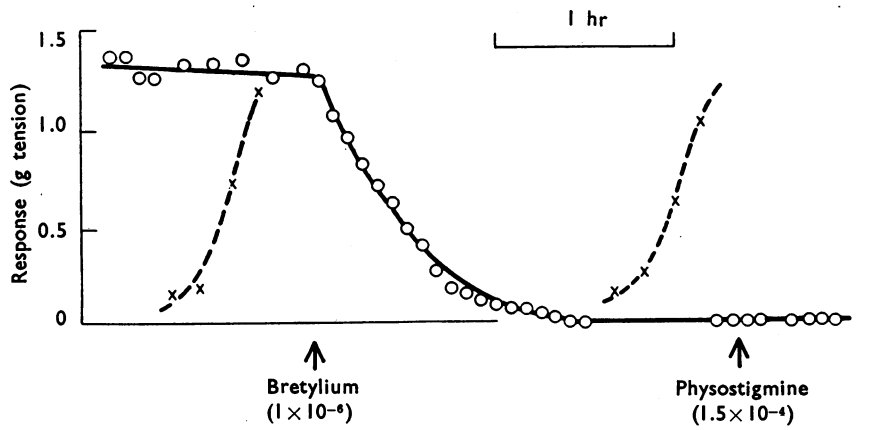

Fig. 3. Effect of bretylium on the responses in isometric tension of the rabbit isolated pulmonary artery preparation to nerve stimulation (200 stimuli at 25 shocks/sec, continuous line) and to acetylcholine (broken line). Sequences of acetylcholine concentrations (2, 6, 20 and 60×10^{-8}) were repeated, each dose being washed out after 1 min of contact. Points are means from two experiments. The abscissa time scale is shown on the top of the diagram.

isolated preparation from a normal rabbit in a bath concentration of 2 to 10×10^{-6} for 2 to 3 hr abolished the response to nerve stimulation, whether or not physostigmine (1.5×10^{-4}) was present. In contrast, the responses to direct stimulation and to acetylcholine were similar in size to those in preparations from rabbits not treated with reserpine.

DISCUSSION

Recently, Burn (1963) has summarized the evidence for the concept that acetylcholine is an intermediary in the release of noradrenaline in postganglionic sympathetic nerves. The more important lines of evidence for certain tissues are given below and are discussed in relation to our results.

(1) Exogenous acetylcholine caused a sympathomimetic effect in the presence of atropine. This "nicotinic" indirect action disappeared after catechol amine depletion by administration of reserpine or by denervation. (2) A cholinergic response was observed on stimulating sympathetic nerves after treatment with reserpine, and was blocked by atropine or hyoscine and potentiated by physostigmine. This response was attributed to a direct action of endogenously liberated acetylcholine on the effector cells. (3) Acetylcholine, hemicholinium and bretylium blocked the effector response to sympathetic nerve stimulation. These compounds were considered to act by interfering with an intermediary cholinergic mechanism. (4) Physostigmine, neostigmine and hyoscine exerted a greater influence on the responses at lower than higher stimulus frequencies of sympathetic nerve stimulation (Burn & Weetman, 1963 ; Burn, Rand & Wien, 1963 ; Burn, Dromey & Large, 1963).

The present work employed the rabbit isolated pulmonary artery preparation, the motor nerve to which, the right recurrent cardiac, had been shown on pharmacological grounds by Bevan (1962) to be postganglionic sympathetic in function. In this preparation, no sympathomimetic effect of exogenous acetylcholine could be demonstrated: the contractile response to acetylcholine was readily blocked by atropine but was unaffected by bretylium, yohimbine and reserpine. All these findings suggest the purely direct, "muscarinic" nature of the acetylcholine action and, hence, the absence of any indirect action (transmitter release). The latter conclusion undoubtedly questions the possible role of acetylcholine on pulmonary artery muscle, if it were released at the sympathetic nerve terminal. Nerve stimulation was totally without effect after treatment with reserpine, even when the tissue had been soaked in physostigmine to potentiate the effect of any acetylcholine which might be liberated. Moreover in the presence of acetylcholine, physostigmine, atropine or hemicholinium, the normal preparation showed no effect attributable to the postulated cholinergic link (such as modification of the responsiveness of sympathetic nerve stimulation or of the stimulus frequency/response relationship) when these agents were applied in concentrations similar to or higher than those found effective in some other tissues in similar studies.

As the reductions in the responses to nerve and direct muscle stimulation were similar after exposure to high doses of atropine and hemicholinium, and the action of the latter drug was not reversed by choline, these effects are unlikely to be due to interference with a cholinergic mechanism.

Burn (1963) suggested that there might be large amounts of noradrenaline in blood vessels which could mask the direct action (on effector cells) of acetylcholine released upon sympathetic postganglionic nerve stimulation. Nevertheless, this direct action would be expected to appear when the catechol amines were depleted or their effect blocked, or when the cholinesterase was inactivated; yet it did not in our experiments. As the effects of bretylium, acetylcholine and other chemically related compounds have been explained by an action on the proposed cholinergic link, these drugs have been considered to reach the site of this mechanism (Burn & Froede, 1963). Thus the present observation that acetylcholine and physostigmine are without effect is unlikely to be due to their poor penetration into this nerve-artery preparation since the latter was readily susceptible to bretylium. Kottegoda (1953) found that acetylcholine had an indirect action on the blood vessels of the rabbit perfused ear. The indirect but not the direct effect disappeared when the ear was skinned. It must be considered that the sympathetic terminals in the blood vessels of the skinned ear, like those in the pulmonary artery, are not responsive to acetylcholine. In view of the high density of chromaffin cells in the skin (Boyd, 1960) and the presence of axon reflexes sensitive to acetylcholine (Coon & Rothman, 1940), evidence obtained from studies of skin circulation is inconclusive.

We conclude that our results provide no support for a mechanism releasing acetylcholine in our rabbit isolated pulmonary artery preparations. We are strongly inclined to the view that the sympathetic postganglionic innervation of the rabbit pulmonary artery is adrenergic in the classical sense.

This work was supported by Grant No. 280 from the Los Angeles County Heart Association. It is a pleasure to thank Dr J. P. Long, Department of Pharmacology, State University of Iowa for his generous gift of hemicholinium, and J. V. Osher for his technical assistance.

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