Br. J. Pharmac. (1970), **38**, 386–393.

The effect of antiadrenaline compounds on acetylcholine responses of frog rectus abdominis muscle

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Summary

1. Nine antiadrenaline compounds and ergometrine have been examined for their effect on acetylcholine (ACh)-induced contractures of the frog isolated rectus abdominis muscle.

2. Tolazoline caused potentiation ($pP_2 = 4{\cdot}14$ at 7 min). Slightly higher concentrations of tolazoline caused antagonism ($pA_2 = 3{\cdot}68$ at 37 min).

3. No potentiation of ACh responses was seen with any of the remaining compounds, which all antagonized ACh responses. Phenoxybenzamine gave the highest, and ergometrine the lowest, pA_2 values.

4. In the concentrations needed to determine pA_2, yohimbine and ergometrine also had other effects on the muscle.

5. These results are discussed in the context of previous findings.

Introduction

Potentiating and blocking actions of antiadrenaline compounds on the responses of isolated guinea-pig vas deferens stimulated either via the hypogastric nerve or by acetylcholine (ACh) have been described (Boyd, Chang & Rand, 1960). These authors suggested that the potentiation was attributable to an anticholinesterase action of the antiadrenalines and showed that these compounds inhibited the destruction of ACh by crude extracts of guinea-pig vas deferens. Their results accorded with other previous observations (Bacq & Fredericq, 1935 ; Jang, 1941 ; Varagić, 1956 ; Huković, 1959) that various antiadrenaline compounds potentiate several sympathetically stimulated preparations. Gowdey (1948) also showed that tolazoline potentiated ACh-induced contractures in both the isolated guinea-pig ileum and frog rectus abdominis muscle, and Burn & Ng (1965) showed that several antiadrenaline compounds potentiated ACh-induced contractures of toad rectus abdominis.

In reviewing the value of experiments using anticholinesterase and antiadrenaline compounds in providing evidence for the participation of acetylcholine in adrenergic transmission, Burn & Rand (1965) pointed to the need for " a quantitative com-

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parison of the anticholinesterase action of physostigmine, neostigmine and several of the antiadrenaline compounds, made in several isolated organs ".

The present work describes the pA_2 (Schild, 1947) and/or pP_2 (Edge, 1967) values obtained with a selection of compounds representative of the main chemical groups with blocking activity at α-adrenoceptors on ACh-induced contractures of frog rectus abdominis muscle. Tolazoline was the only compound of the ten examined with which it was possible to obtain a pP_2 value.

Methods

The methods which have been briefly described elsewhere (Edge, 1967) closely follow Schild's (1947) method of determining pA_x.

Rana temporaria were kept at 4° C until used. Medium sized (15–30 g) animals of either sex but mostly males were killed by decapitation and both rectus abdominis muscles were prepared as described by Burn (1952) and placed in two 4 ml baths containing oxygen-bubbled frog-Ringer solution of the following composition in g/l.: NaCl, 6·50; KCl, 0·14; $CaCl_2$, 0·12; $NaHCO_3$, 0·20; NaH_2PO_4, 0·01. The pH was 8·8. The experiments were performed at room temperature which varied between 19° and 24° C, but in any one experiment did not vary by more than 2° C. The lightly weighted (2–3 g) frontal writing lever had a 5·5 times magnification.

Initially a dose-response regression to ACh was obtained using a dose ratio of two in order to find the dose required to produce an approximately 50% response. This dose was frequently found to differ by a factor of two or more in paired muscles but usually lay between 2 and 8 μg. A 5 min cycle was adopted, ACh being present for 1 min. At the end of each 1 min response, the kymograph was switched off and the bath emptied and refilled twice with fresh frog-Ringer solution. This took 12–15 s. Relaxation of the muscle was manually assisted during the intervening 2·75 min before restarting the kymograph. The kymograph was started 1 min before the next addition of ACh.

The antiadrenaline compound was added, in volumes of 0·05–0·2 ml, to the bath 2 min before the first fractional or multiple (for pP_y or pA_x determinations respectively) dose of ACh and immediately replaced, usually for twelve cycles (57 min contact) after each double wash. To obtain pP_2 or pA_2 values, different concentrations of the antiadrenaline compound were applied to the right and left muscle respectively, and then, after a suitable recovery period, each muscle received the alternative concentration. Ideally this enabled four estimations of pP_2 or pA_2 to be made on one pair of muscles, but if there was an indication of a very persistent effect of the antiadrenaline, only one value was obtained from one pair of muscles. In a few experiments where a change in sensitivity was not considered to be attributable to the persistent action of the antiadrenaline, the doses of ACh were adjusted between the two halves of an experiment to give comparable responses.

Estimation of pP_2

The concentration of the potentiating compound required to potentiate the response of a one-half dose of ACh to match exactly the pre-potentiated control response was estimated by plotting responses (as a percentage of the control) against

concentration of the potentiating compound on semi-logarithmic graph paper. The concentration corresponding to the control (assumed 50% maximal) response was read off. This derived concentration expressed as the negative logarithm of the molar concentration gave a single pP_2 value. Mean values were obtained from several such estimations.

Compounds

In the text, drug concentrations are expressed in terms of the salt. All compounds were dissolved or diluted afresh in frog-Ringer solution, ACh from a 10% w/v solution in ethanol kept at −18° C. The compounds used were: Acetylcholine chloride (Koch Light Laboratories Ltd.); azapetine phosphate (Ilidar, kindly supplied by Dr. A. L. Morrison, Roche Products Ltd.); dihydroergotamine methanesulphonate (ampoules—Sandoz Products); ergometrine maleate and ergotamine tartrate (both kindly supplied by Burroughs Wellcome & Co.); phentolamine methanesulphonate (ampoules—Ciba); and the hydrochlorides of phenoxybenzamine (Smith, Kline & French Laboratories Ltd.), piperoxan and prosympal Rhône-Poulenc, kindly supplied by Mr. A. Crichton, May & Baker Limited), tolazoline (ampoules—Ciba), and yohimbine (Coates & Cooper).

Results

The lowest concentration of each compound tested is shown in Table 1. Except as indicated, each compound was tested in the presence of four repeated constant doses (giving approximately 50% of maximum response) of ACh to find the approximate concentration needed to produce either potentiation or antagonism.

Tolazoline was the only compound which potentiated ACh responses and on which it was therefore possible to determine pP_2 values. With concentrations slightly higher than those needed to determine pP_2 values, however, the potentiated ACh responses began to decline after about four cycles (17 min contact). It was therefore possible to obtain pP_2 values for contact times up to 17 min and pA_2 values with higher concentrations after longer contact times. Recovery from both the potentiating and antagonistic effects was fairly rapid when the drug was removed from the bath so that both pP_2 and pA_2 determinations could be readily obtained in succession on

TABLE 1. *Range of concentrations of antiadrenaline compounds tested on frog rectus abdominis muscle contractures induced with ACh*

Compound	Concentration of salt (μg/ml)	
	Lowest with four constant doses of ACh	Highest
Phenoxybenzamine	0·1*	10
Tolazoline	5·0†	80
Phentolamine	1·0	20
Prosympal	0·025	25
Piperoxan	1·25†	30
Azapetine	0·25	10
Yohimbine	0·1	200
Ergotamine	0·1	25
Dihydroergotamine	0·1	30
Ergometrine	0·025	750

* 5 ng/ml tested in presence of double dose of ACh repeated thirty-six times.
† Not tested in presence of constant dose of ACh.

the same muscle. It was also possible to determine an intermediate concentration which at equilibrium contact time produced neither potentiation nor antagonism of ACh responses, the one effect cancelling the other. This concentration might be considered as $pP_1 = pA_1$. All three values could be determined on one muscle, and one such experiment is illustrated in Fig. 1. The mean results of several determinations are shown in Table 2. The results with tolazoline show that potentiating potency was maximal after 7 min contact ($pP_2 = 4{\cdot}14$), whereas maximal antagonistic potency ($pA_2 = 3{\cdot}68$) required up to 37 min contact (Fig. 1b and Table 2). The difference, $pP_2 - pA_2 = 0{\cdot}46$ shows that only a threefold increase in tolazoline concentration converts a twofold potentiating action into a twofold antagonistic action, although after different contact times. Surprisingly, there was practically no difference between pA_2 and ($pP_1 = pA_1$). In Fig. 1b and c, one concentration of tolazoline was used to determine both values. The mean difference, $pA_2 - (pP_1 = pA_1)$, of 0·07 after 57 min contact (Table 2) was significant only at a P value of 0·4 to 0·5.

The pA_2 values of the remaining compounds are also shown in Table 2. Phenoxybenzamine had the highest pA_2 (6·85) value although the full antagonistic effect

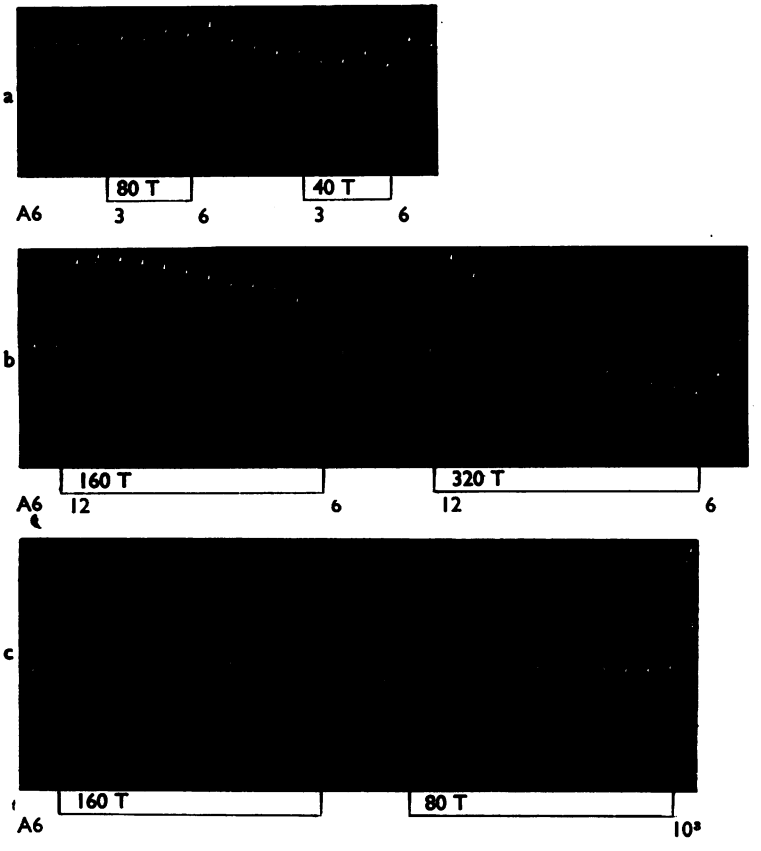

FIG. 1. Left frog rectus abdominis muscle in 4 ml bath. (a), (b), (c), continuous. Effect of tolazoline hydrochloride (T) on acetylcholine chloride (A) contractures induced every 5 min. Doses in μg shown only when changed. At ⊔ , tolazoline hydrochloride (in μg doses shown) added to bath 2 min before subsequent addition of acetylcholine and replaced after each wash. (a), (b), (c) used to determine pP_2, pA_2 and $pP_1 = pA_1$ respectively.

required from 1 to 3 hr contact to develop fully. Phentolamine also had a relatively high antagonistic potency ($pA_2 = 5{\cdot}90$) and, although not displaying any potentiating activity, required a similar contact time to tolazoline to exhibit its full antagonistic action. Azapetine was also relatively potent ($pA_2 = 5{\cdot}15$) and reached equilibrium conditions after contact for only 27 min. Ergotamine and dihydroergotamine had similar pA_2 values but required longer to reach equilibrium. Figure 2 shows that

TABLE 2. *pP_2 and pA_2 values of antiadrenaline compounds on ACh-induced contractures of the frog rectus abdominis muscle*

Compound	Contact time in min	pP_2	$pP_1 - pA_1$ (see text)	pA_2
Phenoxybenzamine	57	—	—	6·58 ± 0·19 (5)
	117	—	—	6·78 ± 0·35 (2)
	177	—	—	6·85 ± 0·21 (3)
Tolazoline	2	4·08 ± 0·09 (14)	—	—
	7	4·14 ± 0·11 (12)	—	—
	17	4·13 ± 0·11 (13)	3·45 ± 0·08 (2)	3·60 (1)
	37	—	3·65 ± 0·17 (3)	3·68 ± 0·17 (7)
	57	—	3·73 ± 0·15 (4)	3·66 ± 0·13 (6)
Phentolamine	17	—	—	5·37 ± 0·21 (7)
	37	—	—	5·72 ± 0·23 (17)
	57	—	—	5·90 ± 0·28 (18)
Prosympal (883F)	17	—	—	4·66 ± 0·22 (5)
	27	—	—	4·70 ± 0·19 (7)
	57	—	—	4·68 ± 0·25 (9)
Piperoxan (933F	17	—	—	4·23 ± 0·02 (2)
	27	—	—	*4·36 ± 0·39 (11)
	37	—	—	†4·46 ± 0·37 (12)
Azapetine	7	—	—	4·83 ± 0·01 (3)
	17	—	—	5·01 ± 0·13 (8)
	27	—	—	5·15 ± 0·13 (10)
	37	—	—	5·15 ± 0·12 (8)
Yohimbine	57	—	—	3·87 ± 0·18 (13)
Ergotamine	57	—	—	4·99 ± 0·15 (6)
Dihydroergotamine	57	—	—	5·13 ± 0·24 (5)
Ergometrine	57	—	—	3·39 ± 0·12 (6)

* Omitting two exceptionally low estimates gives 4·19 ± 0·05 (9).
† Omitting two exceptionally low estimates gives 4·30 ± 0·08 (10).
Values ± standard deviations with number of experiments in parentheses.

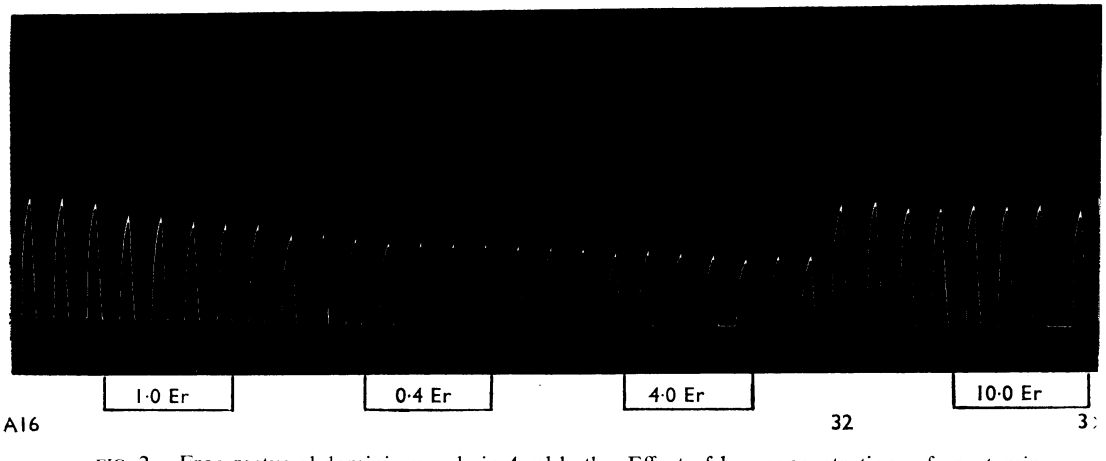

FIG. 2. Frog rectus abdominis muscle in 4 ml bath. Effect of low concentrations of ergotamine tartrate (Er) on acetylcholine chloride (A) contractures induced every 5 min. Doses in μg shown only when changed. At ⌴, ergotamine tartrate (in μg doses shown) added to bath 2 min before subsequent addition of acetylcholine and replaced after each wash.

concentrations of ergotamine from 0·1 to 2·5 μg/ml did not potentiate a constant dose of ACh. Prosympal required only 17 min to give a pA_2 of 4·66 and piperoxan displayed similar characteristics. Yohimbine and ergometrine had very low pA_2 values, 3·87 and 3·39 respectively, comparable with that of tolazoline.

With the high concentrations of the natural alkaloids, particularly yohimbine and ergometrine, necessary to determine pA_2 there was often incomplete relaxation between ACh doses (Fig. 3), so that the pA_2 values for these compounds are probably less accurately determined than the standard deviations would indicate. In some muscles, yohimbine was also observed to cause frothing and slight precipitation in the organ bath. These effects were readily reversible when the alkaloid was removed (Fig. 3b).

Discussion

That tolazoline displayed ACh-potentiating activity is in agreement with the conclusions of Boyd *et al.* (1960) that this compound has appreciable anticholinesterase activity, as was originally shown by Schär-Wüthrich (1943). Boyd *et al.* (1960) also found that higher concentrations of tolazoline antagonized responses to ACh. This also occurred in the present work. The difference, pP_2 (17 min) − pA_2 (37 min) =

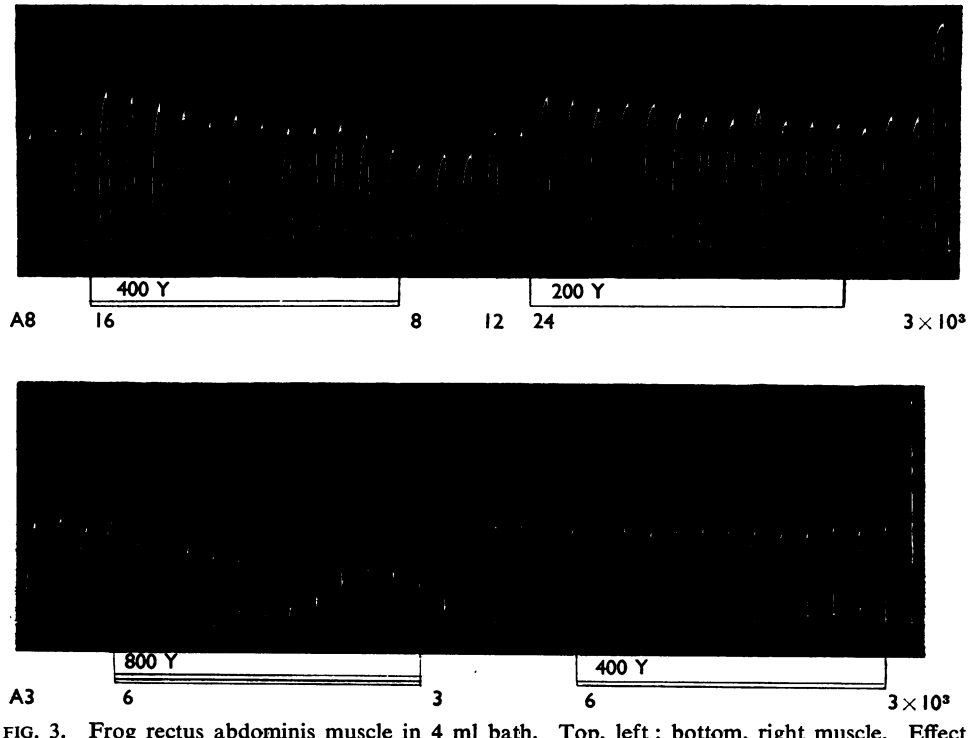

FIG. 3. Frog rectus abdominis muscle in 4 ml bath. Top, left; bottom, right muscle. Effect of yohimbine hydrochloride (Y) on acetylcholine chloride (A) contractures induced every 5 min. Doses in μg shown only when changed. At ⊔, 200 μg; at |—|, 400 μg; at |═|, 800 μg yohimbine hydrochloride added to bath 2 min before subsequent addition of acetylcholine and replaced after each wash. Note that in the right muscle bath only, the addition of both concentrations of yohimbine caused frothing as well as preventing full relaxation between responses.

0·45 ($P<0·05$) and the different times required to produce maximal potentiation (7 to 17 min) and constant antagonism (37 min) might suggest that tolazoline exerts its effect more rapidly on the cholinesterase than on the ACh (nicotinic) receptor of the rectus muscle. It also supports other evidence such as that of Albuquerque, Sokoll, Sonesson & Thesleff (1968) that these two receptors are not identical, although the determination of pA_2 alone does not necessarily imply antagonism at ACh receptors: the blocking action could be at a site beyond the surface receptors.

A potentiating action could not be discerned with any of the remaining compounds, although Boyd *et al.* (1960) found that all of the antiadrenaline compounds examined potentiated the guinea-pig vas deferens response to ACh and inhibited the action of vas deferens cholinesterase. Loewi & Navratil (1926) provided the the first evidence that ergotamine potentiated ACh in the frog heart, and more recently Burn & Ng (1965) have shown a graded potentiation of responses to ACh exhibited by piperoxan, yohimbine and Hydergine on the toad rectus and guinea-pig ileum. It is therefore difficult to reconcile the present findings with those of previous authors, particularly those of Burn & Ng (1965) on the toad rectus. There were, however, technical differences between the two sets of experiments which included the time cycle adopted and the composition and pH of the physiological bathing solution. Cholinesterase activity is maximal between pH 8 and 9 (I. B. Wilson, cited by Nachmanson, 1960) and the anticholinesterase activity of eserine ($pK_a=8·1$) decreases rapidly above pH 8 (Nachmanson, 1960). This could account for the lower pP_2 value obtained by Edge (1967) than that obtained by Smith, Cohen, Pelikan & Unna (1952).

It seems unlikely that high pH could account for the absence, except with tolazoline ($pK_a=10·5$, A. B. Tattersall, private communication), of potentiating activity found in the present work, because azapetine also has a $pK_{a\text{II}}$ of 9·5 (M. W. Parkes, private communication). The pK_a values of the other compounds have not been determined. It might, however, be worth repeating the present work at a more physiological pH.

Another difference between the present experiments and those of Burn & Ng (1965) is that muscles from different species were used. Research initiated by Loewi (1921) showed that vago-sympathetic stimulation of the amphibian heart predominantly caused the release of either " vagusstoff " (ACh) or " acceleransstoff " ([nor] adrenaline), according to the season: the seasonal change in frogs was opposite in toads. Although with the rectus muscle one is not dealing with nerve-induced effects, which in any case are presumably entirely cholinergic, it is not inconceivable that the muscle cholinesterases of the two genera are sufficiently dissimilar for one but not the other to be inhibited by antiadrenaline compounds. However, in an experiment using the toad rectus and 5% CO_2 bubbled solution to lower the pH, piperoxan present at successive concentrations of 2·5, 10 and 40 μg/ml each for four cycles caused only a progressive reduction in the response to a constant dose of ACh. On the other hand, in agreement with Burn & Ng (1965), preliminary experiments have shown that piperoxan exhibits both a potentiating and an antagonistic action against ACh-induced responses of the isolated guinea-pig ileum, in 5% CO_2 bubbled Tyrode solution. It might, however, be of interest to compare the action of the antiadrenaline compounds on cholinesterase extracted from both toad and frog rectus.

I am grateful to Professors E. W. Horton and J. P. Quilliam for their encouragement, interest and helpful criticism, and to Professors W. C. Bowman and J. H. Burn for stimulating discussion of this work, which partially fulfilled the requirements of the Ph.D. degree of London University.

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(*Received November* 6, 1969)