Brit. J. Pharmacol. (1954), **9**, 367.

ATROPINE-LIKE ACTIVITY OF SOME ANTICHOLINESTERASES ON THE RABBIT ATRIA

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(RECEIVED MAY 22, 1954)

The work to be described in this paper is an extension of studies made by Briscoe and Burn (1954) on the ability of quinidine to start the beats of the isolated atria after they had been stopped by acetylcholine (ACh) in the presence of eserine.

Evidence is gradually accumulating to the effect that the action of the group of agents known as anticholinesterases is not due solely to their anticholinesterase activity. In 1949 Quilliam and Strong (1949) showed that eserine and neostigmine had an atropine-like activity on the rabbit heart in that, in the presence of di-*iso*propyl phosphorofluoridate (DFP), they reduced the depression caused by ACh, and abolished the effect of pilocarpine and arecoline. Briscoe and Burn (1954) demonstrated that in high concentrations eserine, neostigmine, and other anticholinesterases—such as the dimethyl carbamate of (2-hydroxy-5-phenylbenzyl)-trimethylammonium bromide (Nu 683); the *N*-*p*-chlorophenyl-*N*-methylcarbamate of *m*-hydroxyphenyltrimethylammonium bromide (Nu 1250); and the dimethobromide of 1:5-di (*p*-*N*-allyl-*N*-methylaminophenyl)-pentan - 3 - one (284C51, Wellcome)—inhibited the action of ACh on the rabbit atria. Neostigmine is known to act other than as an anticholinesterase on skeletal muscle. Thus Riker and Wescoe (1946) found that neostigmine had a stimulant action when all cholinesterase was inhibited, but that DFP had an anticholinesterase action only. McNamara, Murtha, Bergner, Robinson, Bender, and Wills (1954), experimenting with anticholinesterase compounds, suggested that the reversible actions of DFP and TEPP might not result solely from cholinesterase inhibition. Additional evidence supporting this view is found in the comprehensive review of Riker (1953).

Observations now to be described indicate that in high concentrations many anticholinesterases and other substances have an action similar to atropine on the isolated rabbit atria. The contractions of the isolated atria were arrested by ACh in the presence of a low concentration of an anticholinesterase as was described by Webb (1950). Various substances were then tested to see if they would cause the contractions to begin again, as they do when atropine or quinidine is added, as was shown by Briscoe and Burn (1954). Since these workers also demonstrated a close similarity between the action of quinidine and that of a high concentration of eserine, eserine was tried first.

METHODS

Young rabbits of either sex were killed by concussion. The heart was removed and immediately put into cooled Locke's solution at 8–15° C. The Locke's solution contained in one litre 9 g. NaCl, 0.42 g. KCl, 0.24 g. $CaCl_2$, 0.5 g. $NaHCO_3$ and 2 g. dextrose. The atria were dissected free from connective, ventricular and fatty tissues and suspended in a bath of 35 ml., well oxygenated at 29° C.

RESULTS

The results obtained with eserine are illustrated in Fig. 1, which records the contractions of the atria. When eserine sulphate was added to the

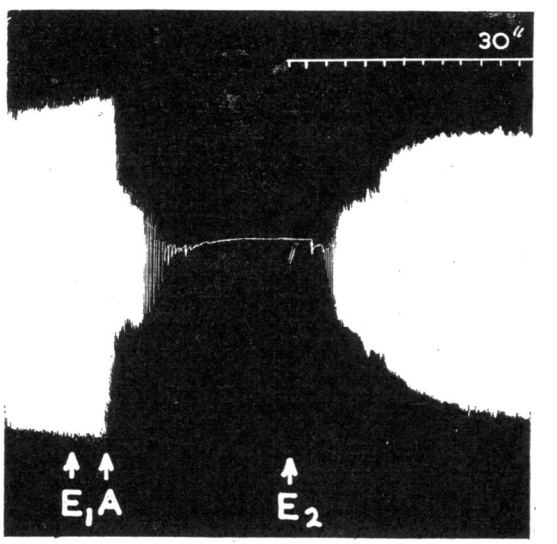

FIG. 1.—Contractions of isolated rabbit atria in 35 ml. bath. At E_1, eserine sulphate 10^{-6} g./ml. At A.1 μg. ACh. At E_2, eserine sulphate, 4×10^{-4} g./ml.

bath to give a concentration of 10^{-6} g./ml., and followed in about 1 min. by 1 μg. ACh, the contractions were arrested, and remained so for about 4 min. The concentration of eserine was then raised so that it was 400 times greater than before. The contractions then began again. This result was observed in 10 out of 15 trials on 4 preparations of isolated atria.

Similar results were obtained with neostigmine, Nu 683, Nu 1250, and 284C51. However, the anticholinesterases which are organic phosphates did not have this action, those tested being DFP, di-ethyl-4-nitrophenylphosphate (E 600 or paraoxon), tetraethylpyrophosphate (TEPP) and bis-(*iso*propylamido)-phosphinic acid anhydride (*iso*-OMPA). Fig. 2 is the record of an experiment

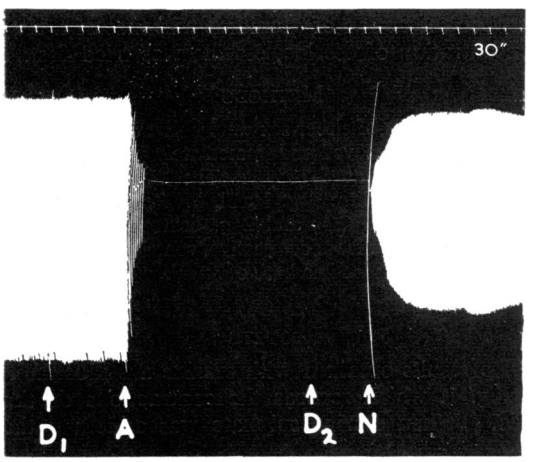

FIG. 2.—Record as in Fig. 1. At D_1, DFP 10^{-6} g./ml. At A, 1 μg. ACh. At D_2, DFP 2×10^{-6} g./ml. At N, neostigmine 4×10^{-4} g./ml.

in which DFP was added to the bath to give a concentration of 10^{-6} g./ml. and, after 2 min., 1 μg. ACh caused arrest of the auricles. A high concentration of DFP (2×10^{-4} g./ml.) was then without effect. A high concentration of neostigmine (4×10^{-4} g./ml.) started the contractions.

A further illustration is shown in Fig. 3, in which E 600 was first used in a concentration of 10^{-6} g./ml., and 5 μg. ACh then stopped the contractions. Concentrations of 2×10^{-4} g./ml. E 600, and then of 4×10^{-4} g./ml., were without effect, but a concentration of 284C51 (4×10^{-4} g./ml.) started the contractions at once. It should be noted that when 5 μg. ACh was then added to the bath it had no action.

Various other compounds which were not anticholinesterases were also tested. Some of these, such as mepyramine, diphenhydramine, procaine,

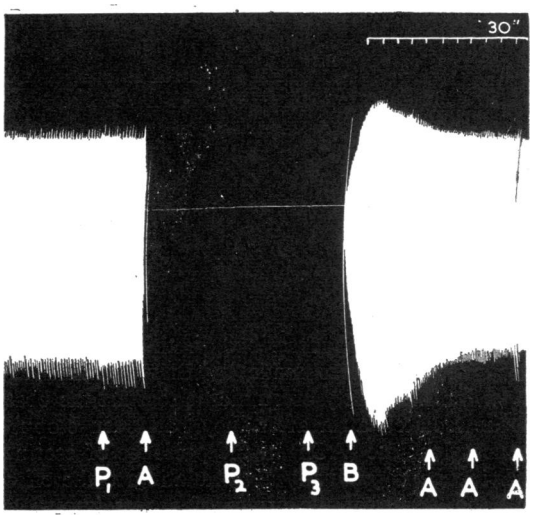

FIG. 3.—Record as before. At P_1, paraoxon 10^{-6} g./ml. At A., 5 μg. ACh. At P_2, paraoxon 2×10^{-4} g./ml. At P_3, paraoxon 4×10^{-4} g./ml. At B, 284C51 4×10^{-4} g./ml.

and pethidine, have been shown to resemble atropine and quinidine, and it was not surprising to find that they also were able to start the contractions which had been stopped by ACh in the presence of a low concentration of an anticholinesterase. Three substances, however, which unexpectedly behaved in the same way were the methyl ester of alanine, decamethonium, and hexamethonium. The two first of these acted rapidly, while the third was slow and feeble in its effect. The substances are shown in Table I.

TABLE I

RESULTS OF TESTS FOR ATROPINE-LIKE ACTIVITY OF VARIOUS COMPOUNDS

The activity tested was the ability to start the beats of rabbits' isolated atria which had been stopped by ACh in the presence of an anticholinesterase

Compound	No. of Atria Used	Proportion of Positive Observations	Dose (mg.) Added to 35 ml. Bath	
			Effective	Non-effective
Eserine	4	10 out of 15	6	—
Neostigmine	1	3 „ „ 3	6	—
Nu 683	1	4 „ „ 4	12	—
Nu 1250	1	3 „ „ 3	12	—
284C51	1	3 „ „ 3	0·2	—
DFP	3	0 „ „ 13	—	0·3–20
E 600	1	0 „ „ 2	—	2–10
TEPP	1	0 „ „ 1	—	5
iso-OMPA	1	0 „ „ 4	—	2–10
Procaine	1	2 „ „ 2	3	—
Pethidine	1	1 „ „ 1	3	—
Diphenhydramine	1	1 „ „ 1	12	—
Mepyramine	1	1 „ „ 1	12	—
Decamethonium	2	2 „ „ 2	5	—
Methyl ester of alanine	2	2 „ „ 2	5	—
N-Acetyl derivative of alanine	2	0 „ „ 2	—	5–7
$CaCl_2$	1	0 „ „ 4	—	10–100
Hexamethonium	5	6 „ „ 10	5–10	—

Discussion

These results add to the evidence that several substances commonly described as anticholinesterases have other actions when they are present in high concentrations. When a low concentration of eserine is added to the bath in which isolated atria are beating, the addition of a small amount of ACh arrests the contractions. A small amount of atropine causes them to beat again. This effect of atropine can be imitated by a high concentration of eserine, or of neostigmine or of other anticholinesterases. It was interesting to observe that the organic phosphate inhibitors of cholinesterase did not have this atropine-like action. The other substances which caused the contractions to begin again were for the most part substances already known to share their properties with atropine (Burn, 1950), but there were two others. One was decamethonium and the other was the methyl ester of alanine. Bülbring (1946) showed that in some respects there was a resemblance between the action of atropine and that of (+)-tubocurarine in the phrenic nerve diaphragm preparation, and the effect of decamethonium and of hexamethonium on the atria may perhaps be considered as atropine-like.

With regard to the methyl ester of alanine, Bergmann, Wilson, and Nachmansohn (1950) have shown that esters of amino acids have an anticholinesterase action. It is therefore interesting to observe that in high concentrations they have an atropine-like action also.

Summary

1. The contractions of the isolated rabbit atria are arrested by small amounts of ACh in the presence of a low concentration of an anticholinesterase. The contractions begin again if a small amount of atropine is added.

2. It has been found that several substances known to have an anticholinesterase action will also cause the contractions to begin again if present in sufficient concentration.

3. Eserine, neostigmine, Nu 683, Nu 1250, 284C51 (Wellcome) have this property, but the organic phosphates such as DFP, TEPP, E 600 and *iso*-OMPA do not.

4. Various other substances which have some atropine-like action also cause the contractions to begin again.

It is a pleasure to express my sincere thanks to Professor J. H. Burn for suggesting this work and for providing facilities in his Department.

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