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Inhibition of succinoxidase activity in normal sarcosomes by sarcosomes from Antimycin-A perfused guinea pig heart

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ANTIMYCIN A is a known inhibitor of the electron transport pathway and would appear to act either on an unknown component between cytochromes *b* and *c* or directly with cytochrome *b* to prevent its oxidation.[1, 2] In previous investigations[4] it was found that perfusion of guinea pig heart with 0·2 μg Antimycin A per ml of Locke–Ringer solution resulted in: (1) early ino- and chrono-tropic negative effects; (2) continued beating of the heart even after 20 min perfusion with Antimycin A, and (3) persistence of negative ino- and chrono-tropism even after perfusing with a medium devoid of antibiotic. The latter behaviour is contrary to that observed for other inhibitors of the oxidative pathway.[3] Since Reif and Potter[5] observed that Antimycin A attached itself firmly to mitochondria for a period of time, it was decided to investigate the behaviour of the sarcosomes of the perfused heart, using succinoxidase activity as a marker of the oxidative pathway, in order to further determine the nature of the effect of Antimycin A on guinea pig heart.

Guinea pig hearts were perfused with 0·2 μg of Antimycin A per ml of Locke–Ringer solution for (a) 5 min; (b) 10 min, and (c) 10 min followed by perfusion for 20 min with Locke–Ringer solution devoid of the antibiotic. Sarcosomes were isolated by the method of Hogeboom[6] from both normal guinea pig hearts and from hearts which had been perfused as described above. In order to ensure that no free Antimycin A remained in the medium, this preparation was washed repeatedly. Sarcosomal protein was determined by the method of Greenberg[7] and succinoxidase activity by the polarographic technique employing the Clark electrode.[1]

Succinoxidase activity in sarcosomes from hearts perfused for 5 min with Antimycin A showed only 10 per cent activity, whilst that from hearts perfused for 10 min showed complete inhibition. Succinoxidase activity was inhibited totally in sarcosomes from hearts perfused for 10 min with Antimycin A followed by perfusion for 20 min with Locke–Ringer solution. These data are in agreement with those of Reif and Potter,[5] and substantiate their suggestion of a binding of Antimycin A by the sarcosomes in the intact heart with the subsequent depression of succinoxidase activity.

The addition of Antimycin A *in vitro* to a normal sarcosomal preparation, in a final concentration of 0·29 μg/mg sarcosomal protein, also inhibited succinoxidase activity (Fig. 1, trace I), in agreement with the data of Ackerman and Potter[8] and of Ahmed *et al.*[9] This was the minimal amount of Antimycin A sufficient to inhibit completely succinoxidase activity under these experimental conditions. If an aliquot of normal sarcosomes was added to an aliquot of Antimycin A-treated sarcosomes, the succinoxidase activity of the combined sarcosome preparations was equivalent to that of the expected activity of the added aliquot (Fig. 1, trace I).

In contrast, if an aliquot of the sarcosomes from Antimycin A-perfused hearts was added to an aliquot of normal sarcosomes, there was an immediate, total inhibition of succinoxidase activity (Fig. 1, trace II). There was a similar total inhibition if an aliquot of normal sarcosomes was added to an aliquot of sarcosomes from Antimycin A-perfused hearts.

Care was taken to ensure that no free Antimycin A was present in the sarcosomal preparation from Antimycin A-perfused hearts. Thus, the observed inhibition of the normal sarcosomes by the sarcosomes from Antimycin A-perfused hearts cannot be explained through the presence of free Antimycin A in the medium. This idea is supported by the finding that *in vitro*-treated sarcosomes do not inhibit untreated sarcosomes although the addition of sarcosomes from Antimycin A-perfused hearts to

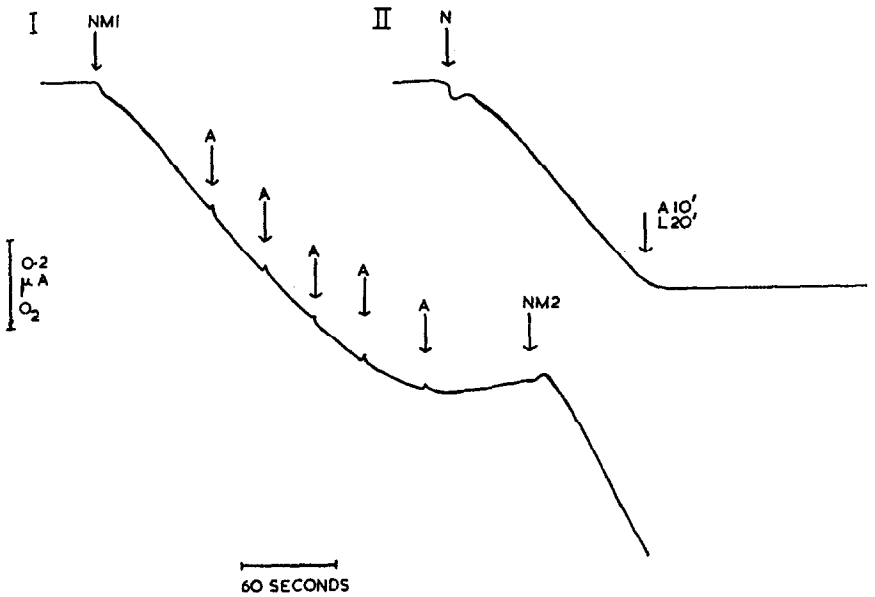

FIG. 1. Rate of oxygen uptake in heart sarcosomes from normal and Antimycin A-treated guinea pigs. The lines (I and II) represent the outputs from an oxygen electrode. Measurements were made at 30°. Normal sarcosomes had an average oxygen uptake of 0·48 ± 0·07 μatoms/min/mg protein with succinate as substrate, and an ADP:O of 1·7 ± 0·3.

I. Normal sarcosomes (NMI = 0·52 mg protein) suspended in 0·25 M sucrose/mM EDTA were added to the reaction mixture (final volume of 0·3 ml) containing 0·25 M sucrose/mM EDTA, 5 mM Na_2HPO_4 (pH 7·4), 5 mM $MgSO_4$, IOmM tris (pH 7·4), 10 mK KCl and 4 mM sodium succinate (pH 7·4). A indicates the addition of 0·03 μg Antimycin A. Total inhibition was achieved with 0·15 μg Antimycin A. NM 2 indicates further addition of normal sarcosomes (0·80 mg protein). II. N indicates addition of an aliquot of normal sarcosomes. A 10 "L20" indicates addition of an aliquot of sarcosomes prepared from guinea pig heart perfused with 0·2 μg Antimycin A/ml Locke–Ringer solution for 10 min followed by Locke–Ringer solution for 20 min. There is an immediate inhibition of succinoxidase activity.

untreated sarcosomes—or vice-versa—resulted in an inhibition of all the succinoxidase activity from untreated sarcosomes. It is possible that this may be due to an interaction at the sarcosomal membrane level, where a transfer of material (Antimycin A?) may occur between inhibited and normal sarcosomes.

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Effect of physostigmine on the level of brain biogenic amines in rats and rabbits

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PHYSOSTIGMINE (eserine) is a reversible inhibitor of cholinesterase and potentiates peripheral as well as central effects of acetylcholine. In both regions its pharmacological actions are different in many ways from other parasympathomimetic drugs such as metacholine or carbamylcholine. The necessity of interpreting the actions of physostigmine solely on the basis of its ability to inhibit cholinesterase has been removed by a series of papers by Varagić and co-workers.[1–3] They concluded that one action of physostigmine is due to a central stimulation of adrenergic nervous elements which is reflected in an elevation of blood pressure after injection of physostigmine to anesthetized rats. They find that acute electrolytic lesions in various parts of the hypothalamus cause a depression or complete abolition of the hypertensive effect of physostigmine. In addition, the blood pressure responses are antagonized by sympatholytic drugs. They feel that a central activation of adrenergic elements take place at a level no lower than the medulla oblongata.

We therefore decided to investigate the effect of large doses of physostigmine on the brain biogenic amines, serotonin and norepinephrine, hoping to demonstrate a direct effect of this drug on these substances.