constant velocity up to about 8 min. The amount of substrate changed is (counts/min in amine spot/total counts/min) × total substrate, and this is taken as a measure of the initial velocity *v*. *Ki* and the type of inhibition can then be found by plotting (1/*v*) against inhibitor concentration.[3] This method of calculation avoids the need to know the counting efficiency or the exact sample size. Figures 1 and 2 are plots obtained by this method showing the competitive inhibition of 5HTP decarboxylation by α-methyl DOPA and the non-competitive inhibition of DOPA decarboxylation by phenylsulphonylhydrazide.

Imperial Chemical Industries, Ltd.,
Pharmaceuticals Division,
Alderley Park,
Macclesfield,
Cheshire, England

A. R. SOMERVILLE

Acknowledgement—It is a pleasure to thank Miss Susan Barnfield for her technical assistance.

REFERENCES

1. W. LOVENBERG, H. WEISSBACH and S. UDENFRIEND, *J. biol. Chem.* **237**, 89 (1962).
2. H. F. SCHOTT and W. G. CLARK, *J. Biol. Chem.* **196**, 449 (1952).
3. M. DIXON, *Biochem. J.* **55**, 170 (1953).

Biochemical Pharmacology, 1964, Vol. 13, pp. 1683–1685. Pergamon Press Ltd., Printed in Great Britain.

The effect of amitriptyline on glycogen phosporylase in cardiac muscle

(*Received* 17 *February* 1964; *accepted* 10 *June* 1964)

INVESTIGATIONS in the peripheral pharmacology of amitriptyline (5-(3-dimethylaminopropylidine)-dibenzo-(a,d)(1-4)-cycloheptadiene) have suggested that this recently introduced antidepressant drug might activate the β- adrenotropic receptor.[1] This conclusion was based on observations made in experiments on the whole animal, on hind limb blood flow and upon the action of amitriptyline on the heart in vivo. In these latter experiments it was demonstrated that amitriptyline potentiated the increase in cardiac output induced by isopropyl nor-adrenaline, but antagonised the increase in output induced by adrenaline.

In view of these observations it was decided to examine the effect of amitriptyline on the activation of glycogen phosphorylase (α-1,4-glucan: orthophosphate glucosyl transferase EC2411) in cardiac muscle. A number of workers have observed that activation of the enzyme phosphorylase depends upon the formation of the substance cyclic 3,5-adenosine monophosphate (cyclic 3,5-AMP).[2] The formation of cyclic 3,5-AMP is promoted by catechol amines; once formed it activates the enzyme phosphorylase kinase which in turn converts "inactive" phosphorylase or phosphorylase-b to "active" phosphorylase or phosphorylase-a. This latter enzyme catalyses the breakdown of glycogen to glucose-1-phosphate. A correlation appears to exist between the increased force of contraction brought about by catechol amines or sympathetic stimulation and the biochemical event, that is, the activation of phosphorylase.[3, 4] This suggestion is further strengthened by the observation that the β-adrenergic blocking agent dichlorisopropyl nor-adrenaline blocks both these actions,[3, 4] and suggests that phophorylase activation may be associated with the β-adrenotropic receptor. Robson and Stacey[5] have proposed that the enzyme catalysing the formation of cyclic 3·5-AMP may be synonymous with the β-receptor.

It was therefore of interest to investigate the action of amitriptyline on heart muscle phosphorylase both alone and in combination with catechol amines.

METHODS

The heart was removed from a freshly killed guinea pig and placed in a watch-glass containing a small quantity of modified Ringer–Locke solution.[6] The ventricles were removed and cut into thin slices, which were then stirred to ensure random distribution of the tissue.

Slices were divided into portions of about 300 mg, which were incubated in 5 ml of modified Ringer–Locke solution which contained the drugs under examination. Two to four portions were used depending on the number of experiments to be carried out, one portion always served as control. Tubes containing the portions were incubated for thirty min at 30°C; they were aerated with a stream of carbogen gas during incubation.

At the end of the incubation period, slices were removed from the tubes and crushed with aluminium tongs pre-cooled in liquid air. Frozen muscle wafers were ground with pestles and mortars which had been pre-cooled in liquid air. The muscle in the solid state was ground into a fine powder; liquid air was added to the mortars during grinding to ensure that no thawing occurred.

The assay method of Cori and Illingworth[7] was modified as follows:

Powdered portions were diluted with 40 vol of an ice-cold solution of 20 mM sodium fluoride, 1 mM ethylene diamine tetra-acetic acid, 0·86% sodium glycerophosphate, and 0·32% cysteine hydrochloride. Resulting homogenates were centrifuged at 4°C for 5 min at 3000 rev/min. 0·5 ml of supernatant was added to 0·5 ml of substrate mixture containing 33·2 mM glucose-1-phosphate, 2% glycogen, and 2 mM AMP, when present. Tubes were incubated for 15 min at 30°C in a metabolic shaker. The procedure was carried out in duplicate, and incubations were terminated by the addition of 1 ml of 0·6 N perchloric acid. Pre-incubation inorganic phosphate (P_I) levels were determined by adding 0·5 ml of extract and 0·5 ml of substrate mixture to 1 ml of 0·6 N perchloric acid. This procedure was carried out with each extract for substrate mixtures with and without AMP. Tubes were centrifuged at 3000 rev/min at 4°C.

P_I determination was carried out according to the method of Berenblum and Chain[8]. Calculation of units of enzyme activity were carried out according to the method of Cori, Cori and Green[9].

RESULTS AND DISCUSSION

The method used has the advantage that test and control tissue are derived from the one heart, and also that the ratio of phosphorylase-a to total phosphorylase (phosphorylase-a + phosphorylase-b) is much less in incubated heart slices than in whole heart. This means that phosphorylase in incubated heart slices has a greater potential for activation than phosphorylase in whole hearts. Thus the ratio of active to total enzyme in heart slices taken immediately *post mortem* is 85·6 per cent; after 30 min incubation this ratio had fallen to 34·4 per cent (mean of four observations). Incubated heart slices however show only about 10 per cent of the total phosphorylase activity seen in fresh whole heart. This may partially account for the need to use high concentrations of catechol amines in order to obtain an activation of phosphorylase in slices.

Initial experiments were conducted to ensure that phosphorylase was activated by adrenaline and isopropyl nor-adrenaline in ventricular muscle slices incubated under our experimental conditions. The only relevant reference to activation in this type of preparation was that of Ellis[10] citing his own unpublished results. The results of five experiments are illustrated in Table 1, in which it can be seen that adrenaline produced a significant ($P<0·05$) activation of phosphorylase in ventricular muscle slices. The effect of isopropyl nor-adrenaline in the same experimental situation is also illustrated in Table 1. The results expressed are the mean of six separate experiments; as in the case of adrenaline there was a significant ($P<0·01$) activation of phosphorylase by isopropyl nor-adrenaline.

The action of amitriptyline on phosphorylase activation is also shown in Table 1. The figures expressed are the mean of eight separate experiments. The control phosphorylase level was 33·5 ($\pm$ 4·2) and this was increased to 47·7 ($\pm$ 4·6) in the presence of amitriptyline (10^{-4} gm per ml). The activation was significant ($P<0·001$).

The effect of isopropyl nor-adrenaline on the activation of phosphorylase in the presence of amitriptyline (10^{-4} gm/ml) was also tested. It may be seen in Table 1 that amitriptyline and isopropyl nor-adrenaline activated cardiac phosphorylase to a similar extent, and that the activation of phosphorylase in the presence of both substances was additive.

In the presence of amitriptyline (10^{-4} gm/ml) the activation of phosphorylase which occurred normally in the presence of adrenaline (10^{-4} gm/ml) no longer took place (Table 1). The results presented suggest that there may be antagonism between adrenaline and amitriptyline; however the variance between the figures was too great to warrant a detailed statistical analysis.

The results demonstrate unequivocally that amitriptyline activates phosphorylase in myocardial muscle slices, and that in this preparation amitriptyline has an action similar to isopropyl nor-adrenaline and adrenaline. The results described demonstrate also, that amitriptyline and isopropyl nor-adrenaline when acting together, enhance the activation of cardiac phophorylase. This action could not be demonstrated for adrenaline in the presence of amitriptyline; indeed, the results obtained suggest that the two substances mutually antagonise the ability of the other to activate cardiac phosphorylase.

TABLE 1. THE EFFECT OF ADRENALINE, ISOPROPYL NOR-ADRENALINE, AMITRIPTYLINE AND COMBINATIONS OF THESE DRUGS ON CARDIAC PHOSPHORYLASE ACTIVITY

Standard control	Adrenaline	Isopropyl Nor-adrenaline	Amitriptyline	Adrenaline + amitriptyline	Isopropyl nor-adrenaline + amitriptyline	P
36·2 ± 3·6 (5)	54·2 ± 6·9 (5)					<0·05
43·0 ± 4·6 (6)		53·7 ± 3·8 (6)				<0·01
33·5 ± 4·2 (8)			47·7 ± 4·6 (8)			<0·001
34·5 (3)	59·0 (3)			30·9 (3)		
47·4 ± 5·9 (4)		57·2 ± 4·5 (4)	57·5 ± 7·4 (3)		67·1 ± 5·7 (3)	

Values given are the percentage ratio of active to total enzyme ± the standard error (number of animals in the group in brackets).

Each line of the table represents a separate comparison.

The P value relates to Student's *t* test of significance. The concentration of drugs was 10^{-4} g/ml free base in each case.

For difference between standard and fresh muscle control see text.

It appears that a distinct parallel exists between the physiological effect of amitriptyline on the heart and the biochemical event. Since the changes in cardiac output appear to be monitored by changes in force of contraction, the results could be extended to support previously proposed correlations between positive inotropic action and activation of cardiac phosphorylase. The mechanism linking the physiological and biochemical event may be the β-adrenotropic receptor.

Department of Pharmacology,
University of Melbourne,
Parkville, Australia.

D. G. SATCHELL
K. D. CAIRNCROSS
S. E. FREEMAN (formerly SIMON)

Acknowledgements—This work was carried out with the aid of a Grant from the National Health and Medical Research Council of Australia. The authors are grateful to Miss I. Lukins for skilled technical assistance.

REFERENCES

1. K. D. CAIRNCROSS, Ph.D. Thesis, *University of Melbourne* (1964).
2. E. W. SUTHERLAND and T. W. RALL, *Pharmacol. Rev.* **12**, 265 (1960).
3. S. E. MAYER and N. C. MORAN, *J. Pharmacol.* **129**, 271 (1960).
4. M. E. HESS, J. SHANFELD and N. HAUGAARD, *J. Pharmacol.* **135**, 191 (1962).
5. J. M. ROBSON and R. S. STACEY, *Recent Advances in Pharmacology*. Churchill, London (1962).
6. M. E. HESS and N. HAUGAARD, *J. Pharmacol.* **122**, 169 (1958).
7. G. T. CORI and B. ILLINGWORTH, *Biochim. biophys. acta*, **21**, 105 (1956).
8. I. BERENBLUM and E. CHAIN, *Biochem. J.* **32**, 286 (1938).
9. C. F. CORI, G. T. CORI and A. GREEN, *J. biol. Chem.* **151**, 39 (1943).
10. S. ELLIS, *Pharmacol. Rev.* **8**, 485 (1956).