Biochemical Pharmacology, Vol. 24, pp. 743–745. Pergamon Press, 1975. Printed in Great Britain.

SHORT COMMUNICATIONS

Cyclic adenosine monophosphate and vascular reactivity in spontaneously hypertensive rats

(*Received* 3 *May* 1974; *accepted* 6 *September* 1974)

A number of studies have shown that beta-adrenergic agonists, as well as some other compounds which cause relaxation of arterial smooth muscle, increase tissue cAMP content either by stimulating adenyl cyclase or inhibiting phosphodiesterase and it has been suggested that a decrease in arterial tone may be mediated by an increase in intracellular cAMP level in the arterial smooth muscle [1–3]. Differences in reactivity between arterial strips from SHR (a strain of spontaneously hypertensive rats developed by Okamoto [4] and used as a model of hypertension) and NR (normotensive rats) suggest that change in some property of the vascular smooth muscle may contribute to the increased peripheral resistance characteristic of essential hypertension [5]. The purpose of this study was to determine whether there are alterations in the cAMP system of vascular tissue from spontaneously hypertensive animals which can be associated with the altered vascular reactivity.

Male Wistar rats of the spontaneously hypertensive strain, 10–12 weeks old, were obtained either from Purina Laboratories or bred in our laboratory. As the SHR were bred out of the Kyoto Wistar strain, Kyoto Wistar as well as Purina Wistar normotensive rats of the same age and sex were used as controls. Blood pressure, measured by a tail plethysmographic method on the day of the experiment, was 118 ± 2·3 mm Hg in NR and 174 ± 2·1 in SHR ($n = 50$). The rats were killed by a blow on the head and the aortas were immediately taken out and carefully cleaned of connective tissue. For the functional studies, two helically cut strips [6] were prepared from each thoracic aorta and suspended under 4 g tension in a tissue bath containing Krebs–Ringer bicarbonate solution (with 10 mM glucose) equilibrated with 95% O_2 and 5% CO_2 to maintain pH at 7·4. Contractions were measured isometrically and recorded on a Grass polygraph via Statham force displacement transducers. After an equilibration period, the effect of relaxing drugs was tested on a half-maximum contraction of the strip induced by serotonin in the presence of phentolamine (1 μM). Preparations from NR and SHR were studied simultaneously under identical conditions. For the assays of enzyme activity, the aortas were homogenized in 50 mM Tris buffer (pH 7·5) at 0° in an all-glass tissue homogenizer. Adenyl cyclase activity was measured by the method of Krishna *et al.* [7] modified by the addition of an ATP regenerating system and by the addition of an excess of cold cAMP to protect the radioactive cAMP formed during the reaction. Phosphodiesterase activity was determined from the decrease in substrate ^{3}H-cAMP at an initial substrate level of 44 μM [8]. The cAMP content of intact tissue was measured in segments of aorta; four segments were obtained from each aorta and randomly distributed in four groups. The samples were incubated in the presence of 10 mM theophylline at 37° for 5 min in Krebs–Ringer bicarbonate medium (with 10 mM glucose) saturated with 95% O_2 and 5% CO_2. At the end of incubation, the tissue was immediately frozen in liquid N_2 and homogenized in ice-cold 5% trichloroacetic acid; cAMP was determined in the supernatant by the protein kinase binding assay of Gilman [9] and protein content in the precipitate by the method of Lowry *et al.* [10].

The basal, nonstimulated, adenyl cyclase activity in aorta of SHR was consistently higher, but not significantly different from that of the control rats (Table 1). There was no difference in adenyl cyclase activity between Kyoto Wistar and Purina Wistar normotensive rats. The sodium fluoride-stimulated adenyl cyclase activity in SHR was significantly higher, by 50 per cent, than in NR. Total phosphodiesterase activity was not different in SHR and NR, and the inhibitory effect of theophylline (2–60 per cent at concentrations of 5–1000 μM) on phosphodiesterase was the same in both groups under the conditions of the assay. The amount of cAMP formed in segments of aorta after the 5-min incubation period was 17·6 ± 0·4 in the NR and 20·7 ± 0·9 pmoles/mg of protein in the SHR ($P < 0.025$). Isoproterenol stimulated cAMP formation in the aortas of both groups of rats. The cAMP dose–response curve to isoproterenol in the aorta of the SHR is shifted to the right of the dose–response curve of the NR; about a five times higher concentration of isoproterenol was required to produce the same increase in cAMP as in NR (Fig. 1). The Lineweaver–Burk and Hofstee plots of the data indicate the same maximum effect of isoproterenol in the aorta of SHR and NR (V_{max} 31·1 ± 2·5; 33·0 ± 4·6 pmoles/mg of protein respectively), while the affinity of isoproterenol appears to be smaller in SHR (K_m 10·2 μM for SHR, 1·55 μM for NR). It was not possible to measure directly the effect of isoproterneol on adenyl cyclase, since adenyl cyclase in vascular tissue loses its responsiveness to catecholamine stimulation in broken cell preparations. There was no difference in the protein content per mg of tissue (w/w) of aorta from NR and SHR.

The relaxing effect of isoproterenol at all concentrations along the dose–response curve was markedly smaller in SHR than in NR; e.g. 1 μM concentration of isoproterenol

Table 1. Adenyl cyclase and phosphodiesterase activity in aorta of normotensive rats (NR) and spontaneously hypertensive rats (SHR)*

Enzyme	NR	SHR	P
Adenyl cyclase			
Basal	19·4 ± 1·8	23·4 ± 2·0	NS
NaF (10 mM)	88·1 ± 5·8	132·7 ± 6·0	<0·001
Phosphodiesterase	719·8 ± 34·9	769·4 ± 42·0	NS

* The enzyme activities are expressed in pmoles cAMP formed or used/mg protein/min. Values are means of 16 animals ± S.E. NS = not significant.

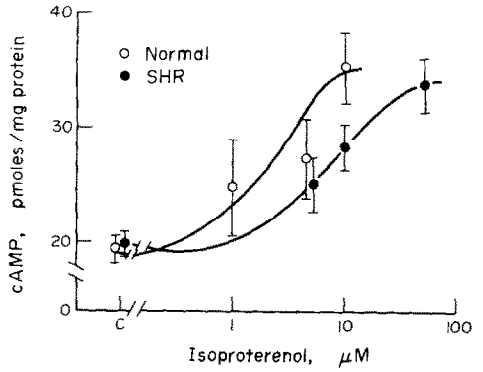

Fig. 1. Effect of isoproterenol on cyclic AMP formation in segments of aorta from NR (○——○) and SHR (●——●). Each point represents the mean of 9 (NR) and 12 (SHR) experiments ± S.E.

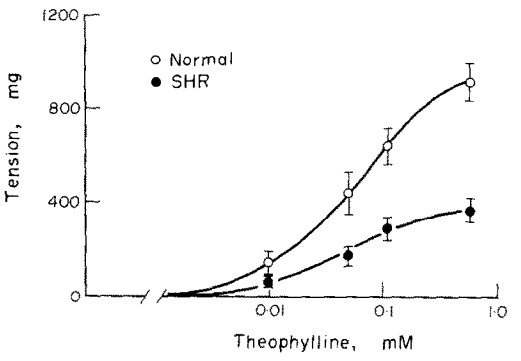

Fig. 3. Relaxing effect (expressed as net decrease in tension) of theophylline on serotonin-induced contraction of aortic strip from NR (○——○) and SHR (●——●). Each point represents the mean of seven experiments ± S.E.

produced 100 per cent relaxation in NR and only 42 per cent relaxation in SHR (Fig. 2). The relaxing effect of theophylline was also significantly smaller in SHR than in NR (Fig. 3), and the dibutyryl derivative of cAMP (0·1 mM) decreased the serotonin-induced contraction of the aortic strip more in NR than in SHR, by 0·58 ± 0·08 g and 0·14 ± 0·06 g respectively.

The present data show that isoproterenol, theophylline and dibutyryl cAMP decrease the contractile response of aortic strip less in SHR than in NR. The cAMP response to isoproterenol in aorta of SHR is smaller and the sodium fluoride-induced adenyl cyclase activity higher than in NR. Some of these results are at variance with those reported by Amer [11], who also observed changes in the cAMP system in aortas of SHR, namely, a slightly higher adenyl cyclase activity, a decreased sensitivity to the stimulatory effect of isoproterenol and less sensitivity to sodium fluoride, while total and low K_m phosphodiesterase activities were increased. Differences in the experimental procedures may account for these discrepancies.

A higher total adenyl cyclase activity (sodium fluoride-induced) in the aorta of SHR indicates a higher amount of the enzyme (sodium fluoride activates the catalytic site of the enzyme and, therefore, maximum response to the compound [10 mM] may be used as an index of the total amount of enzyme [12]). The slightly higher rate of cAMP formation in aorta of SHR may reflect the higher amount of adenyl cyclase activity and could, *per se*, be viewed as a compensatory mechanism to reduce the elevated blood pressure.

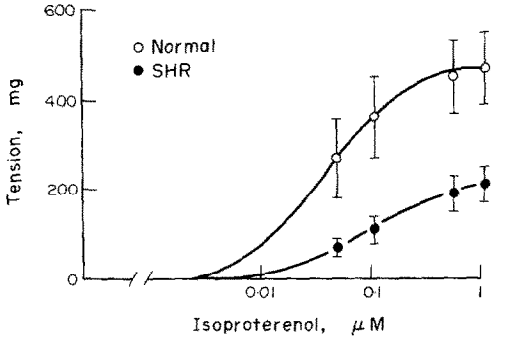

Fig. 2. Relaxing effect (expressed as net decrease in tension) of isoproterenol in the presence of 1 μM phentolamine on serotonin-induced contraction of aortic strip from NR (○——○) (0·50 ± 0·08 g) and SHR (●——●) (0·53 ± 0·09 g). Each point represents the mean of eight experiments ± S.E.

Spector *et al.* [13] have reported that the SHR are "more reactive to the relaxant effects of the stimulant of beta receptors, isoproterenol" and suggested that this "slight enhancement of beta activity may be a compensatory mechanism to reduce the elevated blood pressure". However, in those experiments, isoproterenol was tested against a contractile response which was lower in SHR than in NR and the isoproterenol relaxing effect was expressed as a per cent of the contractile response. Since the amount of relaxation produced by a compound depends upon the degree of contraction [6], the isoproterenol effect was tested in our experiments against a contractile response which was similar in SHR (0·50 ± 0·08 g) and NR (0·53 ± 0·09 g). The relaxing effect of isoproterenol was consistently smaller in SHR and, correspondingly, the cAMP response to isoproterenol in aorta of SHR was decreased, apparently due to a lower affinity of adenyl cyclase for isoproterenol, as indicated by the Lineweaver–Burk plot of the data.

If the higher adenyl cyclase activity found in SHR were only part of a compensatory mechanism to reduce the elevated blood pressure, a higher response to isoproterenol would be expected in SHR than in NR. However, since the affinity for isoproterenol seems to be decreased, the more likely interpretation is that the higher adenyl cyclase activity in SHR may be a compensatory mechanism for the deficient capability of the enzyme to respond to beta-adrenergic stimulation and for the decreased responsiveness to cAMP in SHR, as indicated by the smaller relaxing effect of dibutyryl cAMP and theophylline. This interpretation of the results remains to be confirmed by further studies.

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Biochemical Pharmacology, Vol. 24, pp. 745–746. Pergamon Press, 1975. Printed in Great Britain.

Altered plasma creatine phosphokinase activity in vincristine-treated rats

(*Received* 9 *July* 1974; *accepted* 17 *October* 1974)

A side effect of vincristine, when used for treatment of a variety of human malignancies, is the development of muscle lesions which are secondary to nerve damage [1]. In rodents the myopathic features appear to be more severe [2], so that administration of vincristine to rats may be used to study the relationships between morphological and biochemical changes during the development of primary muscle lesions.

A previous study revealed biochemical changes in vincristine myopathy similar to those reported for several naturally occurring hereditary myopathies [3]. A prominent feature of some of these myopathies is a raised creatine phosphokinase activity (CPK; EC 2.7.3.27) in the blood. Consequently, we have measured plasma CPK activity (PCPK) in rats at various intervals following a single i.p. dose of vincristine at 0·6 mg/kg body wt.

Male Wistar rats (Carworth (Europe) Ltd.) weighing initially 275–310 g were injected with vincristine sulphate (Oncovin®, Eli Lilly) at a single i.p. dose of 0·6 mg/kg body wt. Control rats were given an equivalent volume of diluent (supplied by Eli Lilly to dilute vincristine) containing 0·9% NaCl and 0·9% benzyl chloride as a preservative. Although the vincristine treated animals were provided with Oxoid pellets and water *ad lib.* they lost weight during the course of the experiment, with mean losses as follows—Day 1: 12 g, Day 2: 20 g, Day 3: 25 g, Day 4: 33 g and Day 5: 32 g.

Blood was withdrawn at 1, 2, 3, 4 and 5 days after the vincristine injection from the heart under ether anaesthesia into a heparinized syringe and vials. The plasma was separated from the cells within 1 hr and stored at −20° for a maximum of 2 weeks prior to analysis. PCPK activity is stable under these conditions for at least 2 weeks [4].

Different rats were used for each time interval, and the complete experiment was repeated on 3 groups of control and 3 groups of treated animals except for the 5-day sample where 2 different groups of each were used. All injections and blood sampling were performed between 10 and 12 a.m.

PCPK was determined by the method of Worthy *et al.* [5] except that the recommended *p*-chloromercuribenzoate concentration was doubled, since we found that colour formation was erratic at the concentration recommended by these authors. The activity was calculated as international units/l. plasma.

Several workers have reported a non-linear activation of CPK activity with dilution [5, 6]. To minimize errors due to this dilution effect, all plasmas were diluted 1:5 with water prior to analysis. Although this would result in higher values for plasmas from the controls and an underestimation of the enzyme in plasmas with activity above the linear range of the method, we did this in an attempt to standardize comparisons between high and low values of PCPK activity.

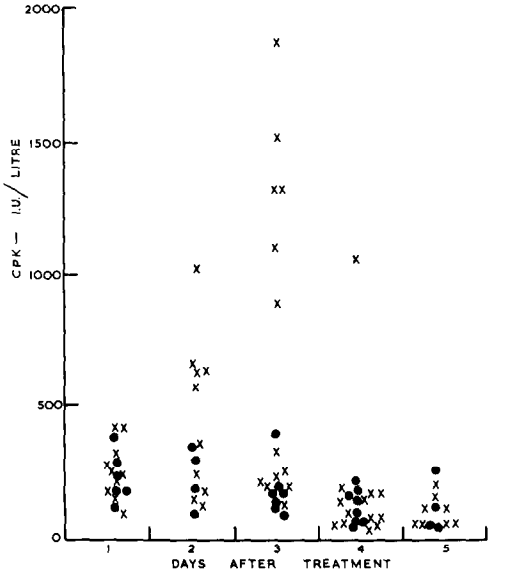

Fig. 1. Distribution of PCPK activities in control (●) and treated (×) rats following a single i.p. dose of vincristine at 0·6 mg/kg. body wt.

The Mann–Whitney *U*-test [7] was used for the statistical comparison of the results obtained 2 and 3 days following treatment. Because of the small number of control samples at the 2-day period, the control values from both these times were combined for this test.

To investigate possible changes in PCPK activity associated with weight loss, 20 control rats were fed a restricted diet so that their body wt at the time of blood sampling was similar to the treated animals, while 9 were fed *ad lib.* No significant difference in PCPK activity was observed between the two groups, the values obtained being 185 ± 100 I.U/l. and 137 ± 72 I.U/l. respectively ($P > 0·2$, Student's *t*-test).

Results are shown in Fig. 1. PCPK activities levels were significantly higher than control values at 2 days ($0·025 > P > 0·01$) and 3 days ($0·01 > P > 0·001$). At both