

those to isoprenaline, a sympathomimetic amine not transported by the neuronal uptake system (Burgen & Iversen, 1965).

Male guinea-pigs weighing 300–350 g were injected intravenously with 6-OHDA,  $2 \times 25$  mg/kg on day 1 and  $2 \times 50$  mg/kg on day 7. The tracheae were removed on days 8–10. Denervation of the tracheae was judged to have been effective as no formaldehyde-induced fluorescence could be detected by histochemical examination and responses to tyramine were abolished.

Isolated, intact tracheae from control or 6-OHDA treated guinea-pigs were set up as described by Coleman & Farmer (1971). In untreated preparations a maximally effective concentration of cocaine ( $10 \mu\text{g/ml}$ —Coleman & Levy unpublished) produced a 3.77-fold ( $P < 0.001$ ) increase in sensitivity to NA but no significant change in sensitivity to isoprenaline ( $P > 0.05$ ). In preparations taken from animals pretreated with 6-OHDA, NA was 6.25 times ( $P < 0.001$ ) more potent and isoprenaline 2.7 times ( $P < 0.001$ ) more potent than in normal tracheae. Cocaine failed to increase further the sensitivity to either NA or isoprenaline in preparations from animals pretreated with 6-OHDA.

The results suggest that the supersensitivity caused by chemical denervation with 6-OHDA resembles that caused by surgical denervation. A post-synaptic component is indicated by (a) the potentiation of responses to isoprenaline in chemically denervated tracheae and (b) the greater potentiation of responses to NA in chemically denervated tracheae than in normal tracheae in the presence of cocaine. A pre-synaptic component is indicated by the greater potentiation of responses to NA than isoprenaline in chemically denervated tracheae.

We wish to thank Mr. G. F. Ainge for carrying out histochemical studies.

#### REFERENCES

- BURGEN, A. S. V. & IVERSEN, L. L. (1965). The inhibition of noradrenaline uptake by sympathomimetic amines in the rat isolated heart. *Br. J. Pharmac. Chemother.*, **25**, 34–49.
- COLEMAN, R. A. & FARMER, J. B. (1971). The inducement of tone and its inhibition in isolated tracheal muscle. *J. Pharm. Pharmacol.*, **23**, 220–222.
- FINCH, L. & LEACH, G. D. H. (1970). Effects of 6-hydroxydopamine on the perfused rat mesentery preparation. *J. Pharm. Pharmacol.*, **22**, 543–544.
- HAEUSLER, G., HAEFELY, W. & THOENEN, H. (1969). Chemical sympathectomy of the cat with 6-hydroxydopamine. *J. Pharmac. exp. Ther.*, **170**, 50–61.
- HAEUSLER, G. (1971). Early pre- and postjunctional effects of 6-hydroxydopamine. *J. Pharmac. exp. Ther.*, **178**, 49–62.
- MALMFORS, T. & SACHS, D. (1968). Degeneration of adrenergic nerves produced by 6-hydroxydopamine. *Eur. J. Pharmacol.*, **3**, 89–92.
- TRANZER, J. P. & THOENEN, H. (1967). Ultramorphologische Veränderungen der sympathischen Nervendigungen der Katze nach Vorbehandlung mit 5- und 6-Hydroxy-Dopamin. *Arch. Pharmak. exp. Path.*, **257**, 343–444.
- TRENDELENBURG, U. (1963). Supersensitivity and subsensitivity to sympathomimetic amines. *Pharmac. Rev.*, **15**, 225–276.

#### The effect of N<sup>6</sup>,O<sup>2'</sup>-dibutyryl adenosine 3':5'-cyclic monophosphate on noradrenaline synthesis in isolated superior cervical ganglia

P. KEEN\* and W. G. MCLEAN

Department of Pharmacology, Medical School, University Walk, Bristol BS8 1TD

Levels of noradrenaline (NA) and its associated enzymes in adrenergic neurones are affected by the activity of the preganglionic fibres (Molinoff & Axelrod, 1971). Pre-ganglionic stimulation causes a several-fold increase in the adenosine 3':5'-cyclic

monophosphate (cAMP) content of the superior cervical ganglion (McAfee, Schorderet & Greengard, 1971). To determine whether this rise in cAMP could play a part in the control of transmitter synthesis we have investigated the effect of N<sup>6</sup>,O<sup>2'</sup>-dibutyryl adenosine 3':5'-cyclic monophosphate (dbcAMP), a stable analogue of cAMP, on the synthesis of NA in rat superior cervical ganglia *in vitro*.

Superior cervical ganglia were removed from albino Wistar rats (250 g) under urethane anaesthesia. The ganglia were desheathed on ice and then incubated at 37° under O<sub>2</sub>/CO<sub>2</sub> in 1 ml of Krebs bicarbonate containing amino acids, vitamins, 3% calf serum and, where appropriate, 1 mM dbcAMP.

Ganglia isolated in this way contained  $17.8 \pm 1.3$  ng NA. After 2 h incubation the NA content of control ganglia was  $23.0 \pm 1.8$  ng ( $n=8$ ) and of dbcAMP-treated ganglia  $42.9 \pm 4.1$  ng ( $n=8$ ), an increase of 87% over the control ( $P<0.01$ ). These levels were maintained for at least 8 h. Theophylline (1 mM) and cAMP (5 mM) also caused a rise in NA content. Cycloheximide (20 µg/ml) did not block the effect of dbcAMP on NA levels showing that this effect did not require the synthesis of new protein.

If, 2 h after the start of incubation when NA levels had reached a new steady state, NA synthesis was stopped by addition of  $\alpha$ -propyldopacetamide ( $10^{-4}$ M), NA levels in control and dbcAMP-treated ganglia declined at the same rate ( $t_{1/2}$  approximately 4 h) showing that dbcAMP did not produce its effect by slowing the release or breakdown of NA. This suggested that dbcAMP might be acting by increasing NA synthesis. To test this <sup>14</sup>C-L-tyrosine was added to the incubation medium 2 h after the commencement of incubation which was continued for a further 1 h. The ganglia were homogenized and the <sup>14</sup>C-dopa, -dopamine and -NA formed were separated by adsorption to alumina followed by differential elution from Amberlite CG-120 resin. In dbcAMP-treated ganglia the <sup>14</sup>C-NA synthesized was  $5.75 \pm 0.62$  (nmoles/g wet wt)/h ( $n=11$ ) as compared with  $3.11 \pm 0.39$  (nmoles/g)/h in control ganglia ( $n=12$ ) an increase of 85% ( $P<0.01$ ).

These results suggest that cAMP may play a role in the short-term control of NA synthesis in the cell body of the sympathetic neurone.

W. G. McLean is a Wellcome Trust Research Training Scholar.

#### REFERENCES

- McAFEE, D. A., SCHORDERET, M. & GREENGARD, P. (1971). Adenosine 3',5'-monophosphate in nervous tissue: increase associated with synaptic transmission. *Science, N.Y.*, **171**, 1156-1158.  
MOLINOFF, P. B. & AXELROD, J. (1971). Biochemistry of catecholamines. *A. Rev. Biochem.*, **40**, 465-499.

### The influence of growth and of adrenalectomy upon some rat heart enzymes

B. A. CALLINGHAM and LAURA DELLA CORTE\*

*Department of Pharmacology, University of Cambridge*

Monoamine oxidase (MAO) activity in the rat heart increases with the age of the animal (Horita, 1968), and also, together with NADH<sub>2</sub> cytochrome C reductase (NCR), following adrenalectomy (Callingham & Della Corte, 1971). In this study the effects of these factors on the half-lives of both enzymes have been examined.

Male Wistar rats were used. They were adrenalectomized under ether anaesthesia and maintained on 0.9% sodium chloride solution. MAO assays were per-