situations stimulation occurred in the cortex. Whereas in uraemia the response of adenylate cyclase to dopamine was found to be inhibited in neostriatum[1], in the cases of jaundice this response was increased.

Elevated levels of diazo-reactive substances in jaundice are due to bilirubin and its derivatives as well as phenols. However similar elevations of diazo-reactive substances were found in uraemia and in this case it seems certain that these represent phenols. Regardless of the nature of these diazo-reactive substances, their levels in brain do correlate very well with the changes in the response of adenylate cyclase. Thus it seems likely that both unconjugated bilirubin and phenols are responsible for these changes in the brain.

1 G.A. Turner and E.N. Wardle, Clin. Sci. molec. Med. *55*, 271 (1978).
2 A.G. Gilman, Proc. nat. Acad. Sci. *67*, 305 (1970).
3 E.N. Wardle and K. Wilkinson, Clin. Nephrol. *6*, 361 (1976).
4 Editorial, Br. med. J. *2*, 143 (1977).
5 E.N. Wardle, Kid. Int. *8*, suppl. S 13 (1978).

The effect of proctolin on the adenylate and guanylate cyclases in the *Locusta* brain at various developmental stages

L. Hiripi, K.S.-Rózsa and T.A. Miller

Biological Research Institute of the Hungarian Academy of Sciences, 8237 Tihany (Hungary), and University of California, Division of Toxicology and Physiology Department of Entomology, Riverside (Ca. 92502, USA), 1 February 1979

Summary. Proctolin at concentrations 10^{-8}–10^{-7} M elevated by 40% brain adenylate cyclase activity of adult *Locusta migratoria migratorioides* R.F. In moulting individuals, proctolin caused a decrease in brain adenylate cyclase activity, and it proved to be ineffective in the larvae. Proctolin caused only a slight decrease on guanylate cyclase activity of the brain at every developmental stage.

For invertebrates, there is growing interest in peptides as transmitter substances or modulators of neuronal activity. Recently[1], it was reported that in Molluscs peptide-containing extract of nervous system can activate adenylate cyclase, although a final determination of this peptide has not been given. One of the peptides which has been isolated and identified from insects is proctolin, a pentapeptide, which has the following amino acid sequence: Arg-Tyr-Leu-Pro-Thr[2]. Proctolin is a putative excitatory synaptic transmitter at the efferent pathway of the proctodeal innervation of *Periplaneta americana* L.[2,3]. Its presence was also demonstrated in the central nervous systems of 6 different species including *Locusta*[4]. The site of action of proctolin was suggested to be at the postsynaptic muscle membrane[3], which in turn suggested the involvement of a second messenger systems in the proctolin effect.

In the present report, the effect of proctolin on adenylate and guanylate cyclase activity as well as connections between this peptide transmitter and cyclic nucleotides at various developmental stages of *Locusta migratoria migratorioides* R.F. will be described.

Methods. Proctolin was tested on adenylate and guanylate cyclase activity at concentrations 10^{-9}–10^{-4} M. The effect was estimated in the brains of 5th instar larvae, 4–5 days before the adult ecdysis, in the brains of moulting animals and in the brain of adult animals 7 days after adult ecdysis. The preparation of homogenate and the composition of the incubation mixture for estimation of cyclase activity was described elsewhere[5]. The production of cyclic AMP was measured by protein binding assay[6] using the cyclic AMP assay kit from the Radiochemical Centre, Amersham. The production of cyclic GMP was measured by radioimmunoassay method (Cyclic GMP, RIA Kit, Product Information; The Radiochemical Centre, Amersham).

Results and discussion. As can be seen in figure 1, the adenylate cyclase activity of larval brain was not significantly changed under the influence of proctolin. In the brain of moulting individuals, the activity of adenylate cyclase was slightly decreased (figure 1). The largest response to proctolin was found in the brain of adult *Locusta*, where the activity of adenylate cyclase was elevated 40% over the control value (figure 1). The maximal effect of proctolin appeared at 10^{-8}–10^{-7} M concentrations in adult and moulting forms alike, although in the first case it caused an increase, and in the second case a decrease in the enzyme activity. At high concentrations (10^{-4} M), proctolin failed to alter the adenylate cyclase activity (figure 1).

The effect of proctolin on the guanylate cyclase was less definite. It caused a slight decrease in guanylate cyclase activity in larval and adult brains, but the degree of inhibition never exceeded 25% of the control value (figure 2). No activation of guanylate cyclase was observed under the influence of the proctolin. The most effective concentration of proctolin was 10^{-5} M in changing the guanylate cyclase activity (figure 2).

Numerous transmitters and hormones of vertebrates are known to alter adenylate and guanylate cyclase activity[7].

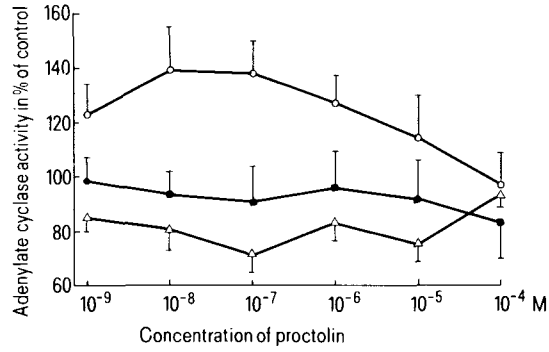

Fig. 1. Effect of proctolin on the adenylate cyclase activity. Each point is the mean ± SD of 4 determinations. Larvae: ●——●; control activity: 53.8 ± 9.4 pmoles cAMP/mg prot./min. Moulting: –△–; control activity: 55.4 ± 6.2 pmoles cAMP/mg prot./min. Adult: ○——○; control activity: 64.2 ± 10.7 pmoles cAMP/mg prot./min.

Monoamines have been known to modify the cyclase activity in different invertebrate phyla, too[1,5,8-10]. Proctolin is the first identified peptide transmitter which has been shown to activate adenylate cyclase in the insect brain. Proctolin also caused an increase in the heartbeat rate of *Periplaneta americana* at concentrations below 10^{-9} M[11]. The adenylate cyclase of *Locusta* brain can be activated by monoamines, too; among them octopamine proved to be the most effective[5]. Comparing cyclase activation caused by monoamines or proctolin, it became obvious that proctolin had lower potency; however, sensitivity increased during development for both types of substances. The failure to activate guanosine cyclase by proctolin suggested the presence of separated receptor sites for these enzymes.

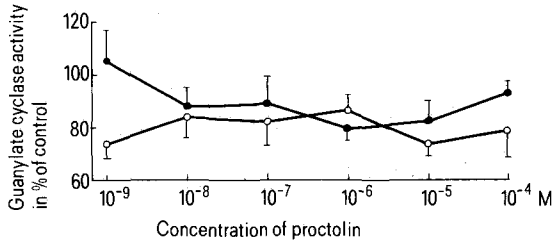

Fig. 2. Effect of proctolin on the guanylate cyclase activity. Each point is the mean ± SD of 4 determinations. Larvae: ●——●; control activity: 32.9 ± 4.1 pmoles cGMP/mg prot./min. Adult: ○——○; control activity: 41.7 ± 4.8 pmoles cGMP/mg prot./min.

1 I.B. Levitan, Brain Res. *154*, 404 (1978).
2 A.N. Starratt and B.E. Brown, Life Sci. *17*, 1253 (1975).
3 B.E. Brown, Life Sci. *17*, 1241 (1975).
4 B.E. Brown, J. Insect Physiol. *23*, 861 (1977).
5 L. Hiripi and K.S.-Rózsa, in preparation (1979).
6 A.G. Gilman, Proc. natl Acad. Sci. USA *67*, 305 (1970).
7 T.W. Rall and A.G. Gilman, Neurosci. Res. Prog. Bull. *8*, 347 (1970).
8 M. Wollemann and K.S.-Rózsa, Comp. Biochem. Physiol. *51C*, 63 (1975).
9 R.C. Filburn and G.D. Wyatt, J. Insect. Physiol. *22*, 1635 (1976).
10 A.J. Harmar and A.S. Horn, Molec. Pharmac. *13*, 512 (1977).
11 T. Miller, Am. Zool., in press (1979).

Stereospecificity of hydrogen transfer of aldehyde reductase

B. Wermuth, J.D.B. Münch and J.P. von Wartburg

Medizinisch-chemisches Institut der Universität, Bühlstrasse 28, CH-3012 Bern (Switzerland), 8 June 1979

Summary. Aldehyde reductase from human liver catalyzes the hydrogen transfer from the pro-4R position on the dihydronicotinamide ring of the coenzyme to the *re* face of the carbonyl carbon atom of the substrate.

Aldehyde reductase from human liver is an NADPH-dependent monomeric oxidoreductase of broad substrate specificity. Several of its physical and chemical properties have been examined, and the enzyme has been shown to bear similarities to other low molecular weight reductases as well as to some oligomeric dehydrogenases[1,2]. These studies suggested an evolutionary link between the monomeric aldehyde reductase and the oligomeric dehydrogenases. One of the most highly conserved characteristics of a given dehydrogenase is its stereospecificity, and it has been contemplated that studies on the stereospecificity may reveal common features among the dehydrogenases having the same stereospecificity. In this work we have determined the stereospecificity of human liver aldehyde reductase and have compared it with that of other aldehyde reductases as well as with that of the oligomeric alcohol dehydrogenase.

Experimental procedure. 4S-(4-^{3}H) NADPH (B-NADPH) was prepared enzymatically with hexokinase and glucose-6-phosphate dehydrogenase (Boehringer Mannheim, FRG) from (1-^{3}H) glucose (New England Nuclear, Dreieichen, FRG) and $NADP^+$. The reaction mixture consisted of 0.1 M Tris-Cl, pH 7.5, 0.33 mM D-(1-^{3}H) glucose (18 Ci/mmole), 0.3 mM $NADP^+$, 5.5 mM ATP, 6.5 mM $MgCl_2$, 5 units of glucose-6-phosphate dehydrogenase and 2 units of hexokinase. The progress of the reaction was monitored by the increase in absorbance at 340 nm. Upon completion of the reaction unlabeled NADPH was added and the mixture was diluted with water to a conductivity of 5 mS and applied to a column (20 × 2 cm) packed with DEAE-Sepharose equilibrated against 20 mM Tris-Cl pH 8.0. 4S-(4-^{3}H) NADPH was eluted with a gradient 0–400 mM NaCl in 20 mM Tris-Cl, pH 8.0.

(4-^{3}H) $NADP^+$ was obtained by stereospecific oxidation of 4S-(4-^{3}H) NADPH with alcohol dehydrogenase. The reaction mixture consisted of 0.01 M Na phosphate, pH 7.0, 0.25 mM 4S-(4-^{3}H) NADPH, 2.5 mM 4-nitrobenzaldehyde and 20 units of horse liver alcohol dehydrogenase (Boehrin-

Stereospecificity of hydrogen transfer reactions of aldehyde reductase

Reaction observed			Specific activity Donor	Acceptor	Percent transferred
			cpm/μmole	cpm/μmole	%
4S-(4-^{3}H) NADPH	AR →	$NADP^+$	$3.61 \cdot 10^6$	$3.31 \cdot 10^6$	92
4R-(4-^{3}H) NADPH	AR →	$NADP^+$	$7.84 \cdot 10^4$	$0.016 \cdot 10^4$	0.2
4R-(4-^{3}H) NADPH	AR →	4-nitrobenzylalcohol	$7.84 \cdot 10^4$	$8.0 \cdot 10^4$	102
(1-^{3}H) n-butanol	ADH →	NADH	$4.21 \cdot 10^3$	$3.86 \cdot 10^3$	92

Tritium labeled coenzyme or substrate was incubated in the presence of the enzyme indicated and the corresponding product was isolated from the reaction mixture as outlined in the experimental section. Aliquots of 50 and 100 μl, respectively were mixed with 15 ml Unisolve (Koch Light Laboratories Ltd, Colnbrook, England) and counted for radioactivity. AR, Aldehyde reductase; ADH, alcohol dehydrogenase.