Brain adenylate cyclase activation in the jaundiced rat

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Summary. Adenylate cyclase responses of brain cortex and neostriatum to noradrenaline and dopamine are increased in rats with jaundice in proportion to its severity. In comparing the situation with uraemia, the relevance of phenols has to be considered.

There are many biochemical hypotheses that purport to explain cerebral dysfunction in jaundice due to liver disease. Neuronal adenylate cyclase activity plays a fundamental part in the function of the nuclei of the brain. In a recent study[1] we have shown that uraemia alters the response of rat brain adenylate cyclase to noradrenaline and to dopamine. In view of the similarity between neurobehavioural abnormalities in uraemia and hepatic coma, it was decided to study the brain in jaundice in like manner.

Methods. Homogenates (4% w/v) of the cerebral cortex and neostriatum in ice-cold buffer (Tris-maleate 2 mmoles/l) pH 7.4 with EGTA (2 mmoles/l) were prepared from brains of Sprague-Dawley rats as previously described[1]. Adenylate cyclase activity in a 50 µl aliquot of these homogenates was quantitated by the rise of cyclic AMP in a 7.5 min incubation period after the addition of either noradrenaline (10 µmoles/l) or dopamine (100 µmoles/l) in a volume of 250 µl.

Cyclic AMP was measured by the competitive protein binding method of Gilman[2]. Jaundice was a combined obstructive/hepatocellular type induced by ligating the common bile duct of rats and injecting i.p. 2 days later either carbon tetrachloride (0.15 ml/100 g) or bromobenzene (0.3 ml/100 g) in olive oil. At the time of study, jaundice was of 4 days duration. Sera were obtained at the time of death by cardiac puncture. These sera and the remainder of the brain tissues were stored at -20 °C. Similar samples were available from uraemic rats.

Later, 0.3 ml aliquots of sera and the brain specimens (each about 1 g) were extracted by the Folch technique using first a neutral (2:1) chloroform/methanol-water mixture (20 ml/g of brain) and thereafter extraction of TCA acidified material. Thus the sera and brain tissues could be analyzed for their phenol content by a diazo-reaction[3]. The diazo-reacting material in the neutral chloroform phase, in the acidified chloroform extract, and the phenyl-glucuronides (neutral aqueous phase subjected to beta-glucuronidase hydrolysis) were assayed separately.

Results. Table 1 presents the results of stimulation of adenylate cyclase in brain homogenates from 15 normal and 13 jaundiced rats (7 caused by carbon tetrachloride and 6 by bromobenzene). Jaundice caused a significant increase in the response of the cortex homogenate to noradrenaline and in the response of the neostriatial homogenate to noradrenaline and to dopamine. Yet the jaundice did not affect the basal unstimulated cyclic AMP concentrations.

Table 2 shows the quantity of diazo-reactive substances found in the extracts of brain and in sera of normal, jaundiced and uraemic rats. There were higher levels of diazo-reactive substances in jaundice in all 3 extracts. Neutral phenols and phenyl glucuronides were particularly high in uraemic samples.

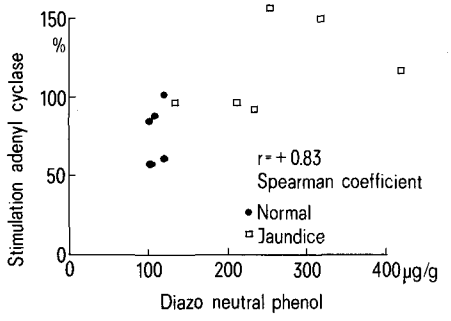
The figures shows the data of tables 1 and 2 presented as the percentage stimulation of adenylate cyclase in the cortex by noradrenaline plotted against the content of diazo-reactive material in the neutral chloroform extracts. This mode of presentation was adopted because of the variable degree of jaundice and of histological liver necrosis in the rats. A positive correlation was found (Spearman coefficient $r = +0.83$) for CCl_4. The same applied to bromobenzene treated rats. Similar correlations were found when the adenylate cyclase response was plotted against diazo-reactive material in the acidified chloroform extracts.

Discussion. There are many potential toxins that might contribute to uraemic and hepatic coma[4]. Phenols are thought to be common toxins in both situations[5]. They are known to produce convulsions and coma. This study has demonstrated that, as in uraemia[1], jaundice can have a marked effect on neurotransmitter stimulation of adenylate cyclase in homogenates of cortex and brain nuclei. In both

Table 1. Percentage stimulation of adenylate cyclase

	Normal N = 15	Jaundice CCl_4 N = 7	Bromobenzene N = 6
Cortex			
Noradrenaline	68 ± 13	117 ± 48	110 ± 24
		p < 0.005	p < 0.01
Neostriatum			
Noradrenaline	44 ± 10	94 ± 47	87 ± 41
		p = 0.001	p < 0.005
Dopamine	62 ± 17	94 ± 17	85 ± 38
		p < 0.001	p = 0.05

Results as mean and SD show within group variation. Statistics by Student's t-test with Bessel's correction for small numbers.



CCl_4 jaundice animals. % stimulation adenyl cyclase and neutral phenol in cortex.

Table 2. Diazo reacting material in serum and brain

	Neutral phase	Acid phase	Phenol glucuronides
Serum µg/ml			
Normal (5)	< 15	< 10	< 5
Jaundice (6)	69 ± 4	56 ± 15	33 ± 8
Uraemia (5)	93 ± 18	66 ± 7	70 ± 7
Brain µg/gm			
Normal (5)	104 ± 16	126 ± 31	185 ± 42
Jaundice (13)	240 ± 60	209 ± 45	195 ± 32
	p = 0.001	p < 0.005	NS
Uraemia (5)	193 ± 21	144 ± 14	269 ± 48
	p < 0.001	NS	p < 0.05

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.

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

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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].

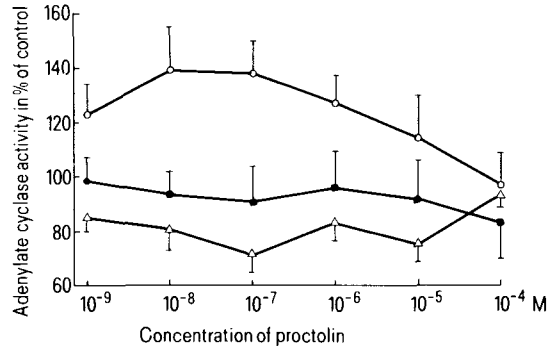

Fig. 1. Effect of proctolin on the adenylate cyclase activity. Each point is the mean ± SD of 4 determinations. Larvae: ●——●; control activity: 53.8 ± 9.4 pmoles cAMP/mg prot./min. Moulting: –△–; control activity: 55.4 ± 6.2 pmoles cAMP/mg prot./min. Adult: ○——○; control activity: 64.2 ± 10.7 pmoles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