Effect of PCPA (100 mg/kg i.p. daily for 4 days) or 5,6-DHT (50 µg into each ventricle) on monoamine concentrations in the hypothalamus and residual brain of the rabbit

Treatment	5-HT (µg/g ± SE)		NE (µg/g ± SE)		DM (µg/g ± SE)	
	Hypothalamus	Rest of the brain	Hypothalamus	Rest of the brain	Hypothalamus	Rest of the brain
Controls	0.97 ± 0.048	0.46 ± 0.026	1.20 ± 0.050	0.30 ± 0.20	0.23 ± 0.010	0.21 ± 0.010
PCPA	0.29 ± 0.010[b]	0.19 ± 0.006[b]	1.07 ± 0.171	0.22 ± 0.008[a]	0.23 ± 0.057	0.18 ± 0.009
5,6-DHT	0.58 ± 0.001[b]	0.30 ± 0.020[b]	1.10 ± 0.120	0.25 ± 0.009	0.28 ± 0.044	0.18 ± 0.010

Each value is the mean ± SE of 6 determinations. [a] $P \leqslant 0.05$; [b] $p \leqslant 0.001$ relative to controls.

Five h after pyrogen administration, the animals were killed by decapitation, the brains were removed and in some cases (see results) the hypothalamus was dissected out. Catecholamines were estimated fluorometrically in the whole brain or in the hypothalamus according to the method of SHELLEMBERGER and GORDON[24]. For 5-HT determinations in blood platelets, some of the animals were exsanguinated under slight ether anesthesia through a polyethylene cannula placed in the carotid artery and blood platelets were isolated as described by DA PRADA and PLETSCHER[25,26]. Significance of differences between groups was calculated by the Student's *t*-test and a probability of $p \leq 0.05$ was accepted as statistically significant.

Results and discussion. PCPA or 5,6-DHT treatment, per se, did not alter the normal temperature of the rabbits; in fact, immediately before pyrogen injection, the basal temperature of the control animals was 39.18 °C, while animals which received PCPA or 5,6-DHT had a mean value of 39.09 and 38.83 °C respectively ($p > 0.05$).

In contrast to the results obtained with PCPA by GIARMAN et al.[11] and MAŠEK, RAŠKOVÁ and ROTTA[12], the Figure shows how, under our experimental conditions, PCPA as well as 5,6-DHT pretreatment was unable to modify the hyperthermic response induced by Pyrifer VII. The levels of the amines were determined in the hypothalamus, in the rest of the brain and, in the case of 5-HT, also in blood platelets, 5 h after pyrogen administration.

The results are shown in the Table: 5,6-DHT reduced the 5-HT content in the hypothalamus and in the rest of the brain to about 60% of the control value, whereas the norepinephrine and dopamine levels were only slightly modified. The central serotonin depletion induced by PCPA treatment was also quite specific and even more pronounced (60–70% depletion). Furthermore, PCPA was able to reduce the 5-HT levels in blood platelets from $97 \times 10^{-6} \pm 10$ to $35 \times 10^{-6} \pm 6$ mmoles/mg protein (64% depletion).

In summary, our results do not seem to support the hypothesis that 5-HT plays an important role in the hyperthermic response induced by pyrogens in rabbits. In fact, a selective reduction of 5-HT hypothalamic levels induced by PCPA or by 5,6-DHT pretreatments, did not modify significantly pyrogen hyperthermia.

[24] M. K. SHELLEMBERGER and J. H. GORDON, Arch. Biochem. *39*, 356 (1971).
[25] M. DA PRADA and A. PLETSCHER, Br. J. Pharmac. *34*, 591 (1968).
[26] M. DA PRADA and A. PLETSCHER, Eur. J. Pharmac. *7*, 45 (1969).

Altered Brain Cyclic AMP-Responses in Rats Reared in Enriched or Impoverished Environments

R. K. DISMUKES[1] and J. W. DALY

National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health Bethesda (Maryland 20014, USA), 2 December 1975.

Summary. The accumulation of radioactive cyclic AMP elicited by various neurohormones has been examined in adenine-labeled telencephalon slices from rats raised in enriched or impoverished environments. Basal levels of cyclic AMP and responses of the brain slice cyclic AMP-generating systems to norepinephrine, isoproterenol and adenosine did not differ between the two group of rats, while responses to prostaglandin E_1 were significantly greater with the impoverished group and responses to histamine appeared to be greater with the enriched group.

Brain cyclic AMP-generating systems have been shown to be capable of adaptive responses to chronic alterations in synaptic input[2]. Thus, when neocortical levels of the neurotransmitter, norepinephrine, are chronically depleted by reserpine, 6-hydroxy-dopamine or lesions of the medial forebrain bundle, the postsynaptic norepinephrine-sensitive cyclic AMP systems of the neocortex become hyper-responsive[3–7]. Similarly, reduction of neocortical levels of histamine and serotonin by lesions of the medial forebrain bundle results in hyper-responsiveness of histamine and probably serotonin-sensitive cyclic AMP-systems in rat cortical slices[7]. Conversely, treatments with drugs such as amphetamine, chlorpromazine, desipramine and imipramine which increase postsynaptic availability of norepinephrine result in a sub-sensitivity of brain slice cyclic AMP-systems to norepinephrine[8–11]. Thus, investigation of the responsiveness of cyclic AMP-generating systems in brain slices would appear to provide a valuable method for probing the effects of chronic drug or environmental manipulations on the synaptic activity of different neurotransmitter pathways in the central nervous system.

Rats raised in enriched versus impoverished environments have been shown to consistently differ in a number

Cyclic AMP formation in telencephalon slices from rats raised in enriched (EC) or impoverished (IC) environments

Agent	No. of pairs of rats	Cyclic AMP (% conversion)	
		IC	EC
None	5	0.26 ± 0.02	0.31 ± 0.09
Norepinephrine	6	3.75 ± 0.41	3.58 ± 0.39
Isoproterenol	6	1.90 ± 0.34	2.36 ± 0.30
Adenosine	6	2.31 ± 0.24	1.99 ± 0.12
Prostaglandin E_1	7	2.24 ± 0.19	1.51 ± 0.27 [a]
Histamine	8	0.58 ± 0.08	0.80 ± 0.12

In 8 separate experiments, telencephalon slices from pairs of EC and IC rats were divided into several portions and incubated with agents for 20 min. All agents were at 100 μM. [a] $p < 0.01$, two-tail matched pair t-test.

of anatomical and biochemical brain parameters (cf. ref.[12] and ref. therein). We have now examined the effects of environmental enrichment versus impoverishment on the responsiveness of brain slice cyclic AMP-generating systems to various neurotransmitters and modulators.

Methods. Male weanling rats (Sprague-Dawley, obtained from NIH) were reared under previously described conditions[12].

Enriched conditions (EC): Groups of 8 rats were housed in wire mesh cages (25″ × 14″ × 10″) and fed ad libitum. Each cage contained 4 'toys' (test tube racks, wire tunnels, jars, wooden blocks, etc.) which were changed daily. 5 days each week the rats were allowed to explore for 30–45 min a Hebb-Williams field maze in which the barriers were changed daily.

Impoverished conditions (IC): Rats were housed individually in opaque cages (11″ × 8″ × 8″) and fed ad libitum. Rats were handled only briefly once a week, while changing litter and adding food. IC rats were kept in same room as EC rats, but in an area screened from direct light.

After 30–60 days of controlled environment, EC and IC rats were sacrificed in pairs; their brains rapidly removed, and the telencephalon chopped on a McIlwain tissue chopper (260 μm spacing). Telencephalon slices were incubated for 20 min at 37 °C in Krebs-Ringer bicarbonate medium aerated with 95% O_2–5% CO_2, collected by pouring over nylon mesh and transferred to medium containing 13 μM [^{14}C] adenine (10 μCi) for an incubation of 45 min. Slices were washed twice, collected on nylon mesh, incubated in fresh medium for 20 min, collected, and divided in equal portions which were placed in separate beakers. Agents under study were added to each beaker and after 20 min incubations with these agents were terminated by collection of slices on nylon mesh and homogenization in cold 5% trichloroacetic acid. The percent conversion of total radioactivity in the slice to radioactive cyclic AMP was determined as previously described[13] except that Dowex and alumina column chromatography as described by SALOMAN et al.[14] was used for isolation of radioactive cyclic AMP. This prelabeling technique with rat brain slices has been shown to give results completely comparable under a variety of conditions to those obtained by measurement of endogenous levels of cyclic AMP[6, 15–17].

Results and discussion. Telencephalon slices were incubated with 5 agents which produce moderate to large stimulation of cyclic AMP formation in rat brain slices. Although no standard environment rats were included as controls in this study, stimulated accumulations of cyclic AMP were similar to those found in numerous prior investigations[3, 6, 7, 15]. No significant differences were found between EC and IC rats in basal levels of cyclic AMP or in the accumulations of cyclic AMP elicited by maximally effective concentrations of norepinephrine, isoproterenol, or adenosine (Table). However, prostaglandin E_1 (PGE_1) produced a 60% greater accumulation of cyclic AMP in IC brain slices than in EC slices. This result was highly significant ($p < 0.01$); in each of the seven separate experiments the IC brain slices were more responsive to PGE_1. The accumulation of cyclic AMP elicited in brain slices from EC rats by PGE_1 appeared comparable to that observed previously with standard environment Sprague-Dawley rats[15]. In contrast, cyclic AMP stimulation by histamine averaged 70% higher in EC than in IC telencephalon slices. However, in both groups of animals, the stimulation produced by histamine was so small that the variability precluded firm conclusions about the significante of this EC versus IC difference. It would appear to the histamine-response in IC rats was comparable to that observed previously in this laboratory with standard environment Sprague-Dawley rats[7].

In the present experiments, cyclic AMP formation was normalized for differences in tissue concentrations by dividing by the uptake of total [^{14}C]radioactivity in each sample. Conceivably, the EC versus IC differences might represent different incorporation of radioactivity into precursor compartments. However, when normalization was instead accomplished by dividing by protein concentration in each sample the same EC-IC differences were apparent (data not shown). Thus, these alterations must represent either enhanced activity of prostaglandin-sensitive adenylate cyclases or decreased catabolism of cyclic AMP in this compartment by phosphodiesterase.

The brains of EC and IC rats have been shown to differ in a number of parameters: density of cortical synaptic dendrites, number and size of synaptic connections, RNA to DNA ratios, glial to neuron ratios, and activities of acetylcholinesterase and cholineacetylase[12]. The mechanisms by which these differences develop and

[1] Present address: Free University, Medical Faculty, Department of Pharmacology, Van der Boechorststraat 7, Amsterdam – Z II, The Netherlands.

[2] R. K. DISMUKES and J. W. DALY, Nature, Lond. submitted.
[3] M. HUANG, A. K. S. HO and J. W. DALY, Molec. Pharmac. *9*, 711 (1973).
[4] A. KALISKER, L. O. RUTLEDGE and J. P. PERKINS, Molec. Pharmac. *9*, 619 (1973).
[5] B. J. WILLIAMS and J. H. PIRCH, Brain Res. *68*, 227 (1974).
[6] R. K. DISMUKES and J. W. DALY, Molec. Pharmac. *10*, 933 (1974).
[7] R. K. DISMUKES, P. GHOSH, C. R. CREVELING and J. W. DALY, Expl Neurol., *49*, 725 (1976).
[8] M. P. MARTRES, M. BAUDRY and J. C. SCHWARTZ, Nature, Lond. *225*, 731 (1975).
[9] A. FRAZER, G. PANDEY, J. MENDELS, S. NEELEY, M. KANE and M. E. HESS, Neuropharmacology *13*, 1131 (1974).
[10] J. VETULANI and F. SULSER, Nature, Lond. *257*, 495 (1975).
[11] J. SCHULTZ, personal communication.
[12] M. A. ROSENZWEIG, E. L. BENNETT and M. L. DIAMOND, in *Macromolecules and Behavior* 2nd edn. (Ed. J. GAITES; Appleton-Century Crofts, New York 1970).
[13] H. SHIMIZU, J. W. DALY and C. R. CREVELING, J. Neurochem. *16*, 1609 (1969).
[14] V. SALOMON, C. LONDOS and M. RODBELL, Analyt. Biochem. *58*, 541 (1974).
[15] K. DISMUKES and J. W. DALY, Life Sci. *17*, 199 (1975).
[16] J. P. PERKINS and M. M. MOORE, J. Pharmac. exp. Ther. *185*, 371 (1973).
[17] A. KALISKER, C. O. RUTLEDGE and J. P. PERKINS, Molec. Pharmac. *9*, 619 (1973).

their functional significance have not been determined. It has been suggested that enriched environments would greatly expand learning experience, and these changes might reflect neuronal alterations associated with learning[12]. The brain slice cyclic AMP system is a logical extension of these studies because its responsiveness has been shown to undergo adaptation to altered synaptic input in vivo. Furthermore, the activity of neurohormone-sensitive cyclic AMP-generating systems would be expected to increase with number of synaptic concentrations. However, most EC-IC differences in brain parameters have been reported to be quite small (< 10%); such small differences in synaptic numbers would probably not be detectable in the brain slice system. The comparatively large (60–70%) alterations seen here in responsiveness of cyclic AMP-generating systems suggests that environmental enrichment may be altering chronic functional synaptic activation of adenylate cyclases responsive to certain putative neurohormones; i.e., prostaglandin and histamine. This possibility would not be unique: alterations in environmental lighting have been shown to control noradrenergic input to the pineal gland, producing corresponding hyper-responsiveness or hypo-responsiveness of post-synaptic cyclic AMP system[18]. Possible correlations of hyper- and hypo-sensitive brain cyclic AMP systems in EC and IC rats with behavioral profiles and drug effects on behavior are under investigation.

[18] J. AXELROD, Science *184*, 1341 (1974).

Brain Glycogen Following Experimental Cerebral Ischemia in Gerbils (*Meriones unguiculatus*)

B. B. MRSULJA, W. D. LUST[1], B. J. MRSULJA, J. V. PASSONNEAU and I. KLATZO

Laboratory of Neuropathology and Neuroanatomical Sciences National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Building 36, Room 4D08, Bethesda (Maryland 20014, USA),
21 November 1975.

Summary. Cortical glycogen levels decreased after both 1 and 3 h of unilateral ischemia. After 1 h of recirculation, the levels of glycogen were restored to control values in both groups. Subsequently, glycogen increased above normal levels after 1 week of recirculation in the 1 h ischemic group, and after 5 h in the 3 h ischemic group. Thus, the onset of the excess glycogen accumulation appears to be dependent on the intensity of the ischemic insult.

Glycogen accumulation in the central nervous system is a common occurrence following a variety of brain injuries. Abnormal deposits of glycogen have been shown in traumatized brains whether the tissue showed histological evidence of injury[2–7] or not[8,9]. Histochemical investigations have demonstrated that the glycogen deposits following brain injury were localized primarily in the astrocytes and neuropil[10,11].

The susceptibility of Mongolian gerbils (*Meriones unguiculatus*) to unilateral ischemia has provided a suitable model for the biochemical investigation of prolonged ischemia, and also the long-term recovery process[12,13]. Using the gerbil model, ITO et al.[14] have shown histologically that certain pathological changes in brain appeared in the post-ischemic period. Further, the evidence indicated that the time of appearance of these histological changes in the post-ischemic period is related to the length of the ischemic insult; the briefer the period of ischemia, the longer the interval before pathological changes occur. The relationship between the duration of ischemia and the development of a detectable lesion has been described as the maturation phenomenon[14].

In this study we demonstrate that the onset of post-ischemic accumulation of glycogen also depends on the duration of the ischemic episode. The results provide biochemical evidence for the existence of the maturation phenomenon.

Materials and methods. Mongolian gerbils (Tumblebrook Farm, West Brookfield, Mass.) weighing 50–60 g were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and the unilateral ischemia was produced by occluding the left common carotid artery with an aneurysm clip. Those animals exhibiting neurological symptoms of cerebral infarction[15] at 1 or 3 h of ischemia were either frozen immediately in liquid nitrogen or at times ranging from 1 h to 1 week after the clip was removed. The outer 2–3 mm of cerebral cortex ipsilateral and contralateral to the occluded artery was excised separately in a cryostat maintained at −20 °C. Sham-operated animals were used for the control levels of glycogen. The frozen tissue was extracted in 0.03 *N* HCl and the glycogen was measured enzymically according to the method of PASSONNEAU and LAUDERDALE[16]. The protein concentrations were determined according to LOWRY et al.[17]. Statistical significance was determined by the Student's *t*-test.

[1] To whom the reprints should be requested.
[2] N. SHIMIZU and Y. HAMURO, Nature, Lond. *181*, 781 (1958).
[3] I. KLATZO, J. MIQUEL, C. TOBIAS and W. HAYMAKER, J. Neuropath. exp. Neurol. *20*, 459 (1961).
[4] L. GUTH and P. K. WATSON, Expl Neurol. *22*, 590 (1968).
[5] M. Z. M. IBRAHIM, E. PASCOE, S. ALAM and J. MIQUEL, Am. J. Path. *60*, 403 (1970).
[6] N. R. CLENDENON, N. ALLEN, T. KOMATSU, L. LISS, W. A. GORDON and K. HEIMBERGER, Arch. Neurol. *25*, 432 (1971).
[7] H. WATANABE and J. V. PASSONNEAU, Brain Res. *66*, 147 (1974).
[8] J. MIQUEL and W. HAYMAKER, in *Progress in Brain Research* (Eds. E. D. P. DE ROBERTIS and R. CARREA; Elsevier, Amsterdam 1965), p. 89.
[9] M. J. MOSSAKOWSKI, D. M. LONG, R. E. MEYERS, H. RODRIQUES DE CURET and I. KLATZO, Expl Neurol. *27*, 500 (1968).
[10] I. KLATZO, E. FARKAS-BARGETON, L. GUTH, J. MIQUEL and Y. OLSSON, Trans. 6th Int. Congr. Neuropath. (Masson et Cie, Paris 1970) p. 351.
[11] A. PRONASZKO-KURCZYNSKA, M. J. MOSSAKOWSKI, M. OSTENDA and J. KORTHALS, Pol. med. J. *10*, 991 (1972).
[12] W. D. LUST, B. B. MRSULJA, B. J. MRSULJA, J. V. PASSONNEAU and I. KLATZO, Brain Res. *98*, 394 (1975).
[13] B. B. MRSULJA, W. D. LUST, B. J. MRSULJA, J. V. PASSONNEAU and I. KLATZO, J. Neurochem., in press (1976).
[14] U. ITO, M. SPATZ, J. T. WALKER, JR. and I. KLATZO, Acta neuropath. *32*, 209 (1975).
[15] K. KAHN, Neurology, Minneap. *22*, 510 (1972).
[16] J. V. PASSONNEAU and V. R. LAUDERDALE, Analyt. Biochem. *60*, 405 (1974).
[17] O. H. LOWRY, N. J. ROSEBROUGH, A. FARR and R. J. RANDALL, J. biol. Chem. *193*, 405 (1951).