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Biochemical Aspects of Protein Synthesis Inhibition by Cycloheximide in One or Both Hemispheres of the Chick Brain

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GIBBS, M. E., A. L. RICHDALE AND K. T. NG. *Biochemical aspects of protein synthesis inhibition by cycloheximide in one or both hemispheres of the chick brain* PHARMAC BIOCHEM. BEHAV. 10(6) 929–931, 1979.—Intracranial administration of cycloheximide into one hemisphere of the chick brain resulted in inhibition of ^{14}C-leucine incorporation into protein only in that hemisphere when the labelled amino acid was administered intracranially. With pericardial injections of labelled amino acid, inhibition of ^{14}C-leucine incorporation was obtained in both the CXM-treated and the untreated hemisphere, when compared with bilateral saline-treated controls. The levels of inhibition were comparable to those obtained with bilateral administration of CXM. There was, however, a slight but significantly higher level of inhibition in the CXM-treated hemisphere. The results were interpreted as supporting the conclusion that monocular learning in chicks resulted in the formation of an engram only in the trained hemisphere.

Unilateral injection of cycloheximide Inhibition of ^{14}C-leucine Incorporation Chickens

PHARMACOLOGICAL and behavioral evidence have been presented to suggest that formation of memory is dependent on protein synthesis [4]. Inhibitors of protein synthesis such as cycloheximide (CXM) and anisomycin inhibit formation of long-term memory in chicks trained binocularly on a single trial aversive discrimination task [3,4]. We have evidence suggesting that in monocular learning memory is formed in the hemisphere contralateral to the eye used in training, since CXM administered to that hemisphere resulted in loss of retention while there was no loss of retention when CXM was injected into the ipsilateral hemisphere [2]. The question arises as to whether CXM in these experiments inhibited protein synthesis only in the hemisphere to which it was administered because Woolston, Morgan and Hambley [7] have put forward data suggesting that unilateral application of CXM reduces lysine incorporation in both hemispheres. This last result would pose a problem for our conclusion that only unilateral engrams were formed following monocular learning. We report experiments investigating the effects of CXM administered to one or both hemispheres of the chick brain on incorporation of leucine.

METHOD

Animals

One or two day-old white Leghorn black-Australorp cockerels were used in all experiments.

Drugs and Reagents

L- (U-^{14}C) leucine (Radiochemical Centre, Amersham) was diluted with 0.9% saline for pericardiac injections and undiluted for intracranial injections. Cycloheximide (CXM, Upjohn Co.) was made up to a concentration of 1 mg/ml with 0.9% saline.

All reagents used were of analytical grade and where appropriate contained 10 mM cold leucine (Sigma). The tissue solubilizer used was NCS (Amersham/Searle).

Procedure

Bilateral experiments. Chicks were injected freehand, using a Hamilton repeating dispenser fitted with a stop, to a depth of 3.0 mm. Ten μl of CXM or saline was administered to each hemisphere 30 min, 1 hr, 2 hr, 3 hr or 4 hr before sacrifice. Five minutes before sacrifice the animals received a pericardiac injection of 1.25 μCi of ^{14}C leucine in a volume of 50 μl.

The animals were then decapitated, their forebrains removed, homogenized and counts in protein and total counts determined as described elsewhere [5]. The samples were counted in a Searle/Nuclear Chicago Scintillation counter (Isocap 300) and results corrected for any differences in quenching.

Results were expressed as:

$$\text{RSA} = \frac{\text{protein counts}}{\text{total counts} - \text{protein counts}}$$

Unilateral Experiments. Chicks were injected in the right hemisphere with CXM or saline in the same manner as for bilateral experiments.

Twenty-five minutes after injection of the drug they re-

TABLE 1

MEAN AND SD OF R S A AND % INHIBITION OF LEUCINE INCORPORATION AT VARIOUS TIMES AFTER BILATERAL ADMINISTRATION OF CXM OR SALINE WITH PERICARDIAL INJECTIONS OF ^{14}C LEUCINE

Time after drug administration (min)	R S.A						Mean Difference R.S A (% inhibition)	t	p
	CXM			Saline					
	Mean	SD	n	Mean	SD	n			
30	0 191	0 088	6	0.739	0 180	5	548 (74.2%)	5 92	<0 001
60	0.184	0.046	6	0 746	0 244	7	562 (75.3%)	5.11	<0 001
120	0.250	0.069	8	0 963	0 111	7	713 (74.0%)	10.55	<0 001
180	0.179	0.037	7	0 486	0 111	8	307 (63.2%)	6 46	<0 001
240	0.283	0.096	7	0 774	0.116	7	491 (63 4%)	7 99	<0 001

n=number of chicks

TABLE 2

MEAN AND SD OF R S A IN RIGHT AND LEFT HEMISPHERES OF CHICKS ADMINISTERED CXM OR SALINE IN THE RIGHT (R) HEMISPERE AND NO DRUG TREATMENT IN THE LEFT (L) HEMISPHERE

Hemisphere and Treatment		Route*	R.S.A.				R.S.A. Mean Difference (L-R)	t	p	n
			R		L					
R	L		Mean	SD	Mean	SD				
CXM	No drug	PC	0 224	0 012	0 245	0.022	0 021	5 25	<0 02	4
CXM	No drug	IC	0 087	0 045	0.218	0 104	0 131	2.63	<0 05	6
SAL	No drug	IC	0.382	0.163	0.290	0 142	-0 092	-1.19	>0.30	6

n=number of chicks

*Route of administration of ^{14}C leucine pericardial (PC) or intracranial (IC)

ceived either a pericardiac injection of 1.25 μCi ^{14}C leucine or an intracranial, bilateral injection of 0.5 μCi of ^{14}C leucine from a Hamilton repeating dispenser syringe set up for drug injections.

The animals were sacrificed 5 minutes after receiving the pulse, and the forebrains removed, divided into the two hemispheres and each homogenized separately. The homogenates were treated as for bilateral experiments except that RNA was removed by digestion in IM NaoH at 37°C and lipid removed by washing in acetone, ether/alcohol and then ether.

Results were expressed as in bilateral experiments.

RESULTS

CXM administered to both hemispheres resulted in significant inhibition of leucine incorporation into TCA precipitable material compared with saline controls (Table 1) regardless of the time at which the assay was carried out. In addition, tyrosine incorporation was also shown to be inhibited by 84.3% 1 hour after CXM administration and another protein synthesis inhibitor, anisomycin produced 68% inhibition of leucine incorporation when measured 30 min after administration. The anisomycin results are comparable to those obtained with CXM. It may be noted that while there is no significant change in inhibition over the first 2 hours, the degree of inhibition decreases slightly after 2 hours. We have no explanation for the relatively low mean R.S.A. value at 3 hours for the saline control group

With CXM administered into one hemisphere only and labelled leucine administered pericardially there was a small but significantly lower level of incorporation in the treated compared to the untreated hemisphere (Table 2). However, the level of leucine incorporation in either hemisphere in these chicks is substantially below that observed for chicks injected bilaterally with saline (see Table 1). It would appear that unilateral administration of CXM with pericardiac injection of labelled leucine leads to inhibition of incorporation in both the treated and the untreated hemispheres. This result confirms the findings of Woolston *et al* [7]: the level of incorporation is comparable to that obtained with chicks treated bilaterally with CXM (see Table 1).

A markedly different pattern of results was obtained when the labelled amino acid was administered intracranially (Table 2). Unilateral administration of CXM produced a significant reduction of the amount of leucine incorporation into TCA precipitable protein in the treated hemisphere when compared to the untreated hemisphere of the same chick. Unilateral administration of saline yielded a level of incorporation not significantly different from that in the untreated hemisphere of the same chicks. However, there is clearly a substantial inhibition of leucine incorporation in hemispheres treated with CXM (mean R.S.A. .087) compared with hemispheres treated with saline (mean R.S.A. .382), while the levels in the corresponding untreated hemispheres were comparable (mean R.S.A.'s .218 and .290).

It is interesting to note that unilateral administration of

saline with an intracranial injection of label yielded levels of incorporation considerably lower than those obtained with bilaterally injected saline chicks administered label pericardially. It is not clear whether this difference is attributable to the difference in the route of administration of the labelled amino acid.

DISCUSSION

The results of these experiments provide conclusive evidence that CXM when administered into one or both hemispheres of the chick brain significantly inhibits leucine incorporation in the hemispheres to which it is administered. Whether or not a unilateral application of CXM will lead to inhibition of incorporation in the untreated hemisphere as well appears to depend on whether the label is administered intracranially or pericardially. With respect to the latter our results confirm those obtained by Woolston *et al.* [7] using labelled lysine, although we used a pulse time of 5 minutes. With intracranial administration of leucine however, inhibition appears to be restricted to the hemisphere treated with CXM.

A possible explanation of the differences obtained between the two routes of administration of the labelled amino acids is that protein or RNA synthesis occurring as a result of learning may be restricted to a specific brain location. Bateson, Horn and McCabe [1] found that increases in incorporation of ^{14}C-uracil in the chick following imprinting occurred in a small area caudal to the mid-point between the anterior and the posterior poles of the forebrain (i.e. in the centre of the forebrain). This appears to be the approximate area into which we inject CXM and the intracranial labelled leucine. It has been pointed out that intracerebral pulse labelling achieves locally a high specific activity [6].

Peripheral administration of label ensures uniform distribution throughout the brain. If protein synthesis associated with memory formation has precise and restricted anatomical localization (Gibbs and Andrew, in preparation) peripheral administrations of label may decrease the likelihood of detecting inhibition of protein synthesis in such an area in whole brain preparations. It may not be surprising therefore that in both our experiments and those of Woolston *et al.* no large differences in inhibition of amino acid incorporation between treated and untreated hemispheres was observed with pericardiac administration, although the small difference we obtained was significant.

Our findings are consistent with behavioral and pharmacological evidence that monocular learning leads to a unilateral engram, the formation of which will be inhibited by protein synthesis inhibitors administered into the trained hemisphere.

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