Effects of Chlorpromazine on Neural Tissue in Culture

Previous studies have shown that chlorpromazine administered to chick embryos at 5 days of incubation significantly increases the activity of acetylcholinesterase (AChE) in the embryonic spinal cord but not that in the cerebral cortex, cerebellum or hypothalamus[1]. The present study was designed further to investigate this differential biochemical response of developing CNS structures to chlorpromazine. The effects of chlorpromazine were studied on the AChE activity and protein content of cerebellar and spinal cord explants removed from chick embryos at 9, 16, and 20 days of incubation and maintained in culture.

Material and methods. Explants of cervical spinal cord were removed from 9- and 16-day-old chick embryos and cerebellar explants were removed from 16- and 20-day-old chick embryos and were maintained as organ cultures according to the technique described previously by VERNADAKIS and TIMIRAS[2]. The culture medium was EAGLE's[3] basal medium with EARLE's[4] balanced salt solution. Freshly prepared culture media with or without chlorpromazine were added to center well of the organ culture dish, 0.5 ml of medium/dish and equilibrated at 35–36° for 18 h prior to transfer of explants. At 18 h of equilibration of the media, explants were oriented on a triangular stainless steel organ culture grid[5], one explant per grid, and were placed in the center well of the organ culture dish. Because chlorpromazine is unstable in solution after 24 h[6], the total experimental period, equilibration plus incubation, was 22 h. Chlorpromazine HCl was prepared in EAGLE's basal medium immediately before equilibration period began. The concentration of chlorpromazine in the medium was 0.08 µg/ml medium or 0.05 µg/mg of tissue. This dose was extrapolated from in vivo studies and was considered of maximal concentration[1]. AChE activity was measured colorimetrically by the method of ELLMAN, COURTNEY, ANDRES and FEATHERSTONE[7] and protein by the FOLIN-LOWRY[8] reaction. To determine whether the parameters measured in control and test explants differed significantly in their mean, the *t*-test for non-paired data was applied[9].

Results. AChE activity was significantly higher in spinal cord explants removed from 9-day-old chick embryos and cultured on basal medium containing chlorpromazine as compared to explants cultured on basal medium only. The enzyme activity did not differ in spinal cord explants removed from 16-day-old chick embryos or cerebellar explants removed from 16- or 20-day-old chick embryos and cultured on basal medium with or without chlorpromazine. Protein content was not changed in explants cultured on basal medium containing chlorpromazine as compared to those cultured on basal medium only.

Discussion. The present results showed that spinal cord explants but not cerebellar explants were sensitive to chlorpromazine in culture. Furthermore, the sensitivity of spinal cord explants to chlorpromazine appeared to be age-dependent. Spinal cord explants from 9-day-old chick embryos, but not those from 16-day-old chick embryos, were sensitive to chlorpromazine.

The spinal cord is morphologically, biochemically, and functionally in an advanced stage of maturation in 16-day-old chick embryos[10]. It has been found previously that AChE activity progressively increases in the spinal cord, reaches peak activity at 14 to 16 days of incubation and remains at this level until hatching time at 20 to 21 days. In contrast to the spinal cord, AChE is very low in the cerebellum of chick embryos at 14 to 16 days of incubation, increases slowly up to hatching time and reaches peak activity at 90 days after hatching[11]. It appeared, therefore, that chlorpromazine affected AChE activity in

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Effects of chlorpromazine on acetylcholinesterase activity and protein content of cultured cerebellar and spinal cord explants removed from chick embryos

Culture[a] medium	Age of chick embryo (day of incubation)	Acetylcholinesterase activity (µmoles AcTCh hydrolyzed/min/g wet tissue)		Protein content (mg/g wet tissue)	
		Cerebellum	Spinal cord	Cerebellum	Spinal cord
Non-cultured	9	–	8.02 ± 0.39[b]	–	27 ± 2
	16	4.70 ± 0.19	18.52 ± 0.48	43 ± 1	51 ± 2
	20	16.42 ± 0.64	–	80 ± 2	–
Basal	9	–	7.39 ± 0.32	–	27 ± 1
	16	4.89 ± 0.22	19.93 ± 0.82	45 ± 1	53 ± 2
	20	16.35 ± 1.0	–	74 ± 3	–
Chlorpromazine	9	–	9.87 ± 0.40 (< 0.001)[c]	–	29 ± 2
	16	5.39 ± 0.27	19.62 ± 0.79	45 ± 2	56 ± 3
	20	16.94 ± 1.31	–	78 ± 3	–

[a] The medium was EAGLE's[3] basal medium with EARLE's[4] salts. The concentration of chlorpromazine was 0.08 µg/ml medium. [b] Each value represents the mean ± SE of 12–20 explants. [c] Numbers in parentheses are *P* values for comparison to basal groups.

the immature spinal cord. The lack of chlorpromazine effect on the mature spinal cord further supports the existing disagreements concerning the effects of chlorpromazine upon adult spinal levels[6].

It can be speculated that the higher AChE activity in the chlorpromazine-treated 9-day-old spinal cord explants may reflect enzyme induction by this drug. Chlorpromazine, like other CNS drugs such as barbiturates, diphenylhydantoin and chlordiazepoxide, has been reported to induce enzymes[6]. Further studies, however, are required in order to propose such a selective action of chlorpromazine on AChE.

AChE activity has been used as evidence for the presence of acetylcholine[12], a proposed neurotransmitter in many CNS areas[13]. Thus, the higher AChE activity in the maturing spinal cord observed in this study and also in previous studies[1] suggests that chlorpromazine influences cholinergic systems of the developing spinal cord. Moreover, chlorpromazine has been found to increase the sensitivity of the developing CNS to seizures elicited by electroshock stimulation in chick embryos[1] and in maturing rats (unpublished observations). Spinal reflex systems play a substantial role in the integration of seizure activity. The influence, therefore, of chlorpromazine on cholinergic systems of the developing spinal cord may be an underlying factor in the sensitivity to electrical convulsions induced by chlorpromazine[14,15].

Résumé. L'activité acétylcholinesterasique de fragments de moelle d'embryon de poulet de 9 jours augmente lorsqu'ils sont maintenus en culture organotypique, après addition de Chlorpromazine au milieu standard de EAGLE[3].

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Radioprotection and Recovery by Dithiothreitol

It is well known that many chemical compounds provide protection against the effects of ionizing radiations in mammals and living organisms. The most effective class of radioprotective molecules seems to be the aminothiols. The presence of both the –SH and $-NH_2$ groups appears to be critical for protection[1]; moreover, a spatial separation between these groups of more than 3 carbon atoms causes a marked decrease in activity[2]. Although dithiothreitol (DTT)[3] bears no $-NH_2$ group, it came to our attention for its high erythrostimulating activity[4], its very low redox potential (– 0.33 V at ph 7) and the presence of its molecule of 2 free –SH groups. In addition, its high solubility in physiological medium and its very low rate of oxidation at room temperature make it a very suitable compound for recovery and radioprotection experiments[5].

Material and method. Male albino mice, Swiss strain, 8 weeks old, weight range 24–27 g, kept unlimited standard diet, were whole-body irradiated in groups of 20 in a 'Perspex' box lying on a wooden support to avoid backscattering. The animals were exposed to an X-ray dose of 625 R by a Siemens Stabilipan unit operating at 180 kV, 10 mA, filtration 0.5 Cu, 26 R/min. In these conditions only $5.6 \pm 3.5\%$ of the untreated animals survived irradiation, the effect being observed in 200 mice. The mortality-rate was checked daily up to the 30th day following X-ray exposure.

Dithiothreitol in the reduced form was purchased from Calbiochem (USA). The trial solution adjusted to pH 7.0 was injected i.p. in each group of 50 animals, at a constant volume of 0.2 ml/mouse. In the protection experiments, the injection was performed 15 min prior to exposure.

Results. Owing to drug toxicity, the highest dose of DTT in the reduced form that could be administered was 120 mg/kg. As shown in Figure 1, this dose causes a moderate survival percentage ($29 \pm 3.4\%$). A dose of 80 mg/kg, however, produces a survival percentage of $15 \pm 1.2\%$; while no radioprotection was detected when lower doses were administered.

In separate experiments (Figure 2), when the 120 mg/kg dose was injected 0.5, 2.5, or 24 h after irradiation, no significant difference in the survival rate could be observed. Only when the compound was administered 72 h after exposure, did survival decrease to $14 \pm 1.0\%$.

From these experiments it was evident that dithiothreitol had a low radioprotective activity and that the same effect could be detected when the drug was given within 24 h after exposure. Since, to our knowledge, no other free –SH groups bearing compound has this particular feature, a question was raised whether the oxidized form of DTT rather than the reduced one accounted for this effect. The former was easily obtained following Cleland's method, by oxidizing the –SH groups to form a disulphide bridge, i.e., producing a ciclization of the molecule.

As shown in Figure 1, when DTT in the oxidized form (200 mg/kg) was injected i.p. before X-ray exposure, survival at 30 days was increased up to $56 \pm 0.6\%$. A dose of 120 mg/kg gave 10% survival, while a higher dose (160 mg/kg) brought the survival to $34 \pm 6.2\%$. No different results were obtained in other experiments (Figure 2) when

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