Studies on the Interaction Between Gamma-Aminobutyric Acid and Tubocurarine on Intraventricular Injection in Cats. (31075)

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Gamma-aminobutyric acid (GABA), a normal constituent of the brain, has been reported to exert an inhibitory action in the central nervous system(1).

During chemically-induced seizures the concentration of GABA in the brain decreases (2) whereas increasing the concentration of GABA in the brain protects against chemically-induced seizures(3).

Intraventricular injection of tubocurarine has been shown to cause convulsive movements which closely resemble epileptic seizures in humans(4). Hence, it was thought to be of interest to explore whether GABA could antagonize the convulsive effect of tubocurarine injected intraventricularly.

Methods and material. Cats weighing 2-4 kg were anesthetized with pentobarbital sodium 35 mg/kg i.p. A Collison intraventricular cannula was implanted aseptically in the lateral cerebral ventricle(5). An artificial cerebrospinal fluid (CSF)(6) was gassed with an O_2:CO_2 mixture (95:5) for 15 minutes. The pH of the resultant solution was 7.35. This solution was used to dissolve the drugs for intraventricular injection. The volume injected did not exceed 0.1 ml. This was followed by 0.1 ml of artificial CSF. All of the solutions used were sterilized.

The drugs used were tubocurarine chloride and gamma-aminobutyric acid. The behavioral effects in 3 unanesthetized cats were observed for 3-4 hours following intraventricular injections of these drugs.

For the drug interaction studies usually the GABA and tubocurarine were injected together. In a few experiments the tubocurarine was injected 10 minutes after injection of GABA. An interval of one week passed between experiments. In the succeeding weeks the tubocurarine or GABA was injected alone and the drug combination was also repeated.

Results. Tubocurarine. The results of the intraventricular administration are listed under Experiment 1 in Table I. The convulsions occurred 30-40 minutes after the injection, then periodically for the next 45 minutes.

During the recovery period there were occasional bouts of loud calling and twitching of the facial muscles, grooming and scratching. These observations are in agreement with those of Feldberg and Sherwood(4). If the animal had received GABA or the combination of GABA and tubocurarine the week before, no preconvulsive phase or convulsions were seen when tubocurarine was administered (Table I, Exp. 2).

GABA. Although a cat which had received an intraventricular injection of 5 mg of GABA appeared to be sedated and apathetic (Table I, Exp. 3), it still responded to tactile stimuli. Doses of 10 and 15 mg GABA caused a greater degree of sedation and catatonia plus respiratory depression as has been previously described by John *et al*(7).

GABA plus tubocurarine. In Exp. 4-6 of Table I the drug combination was given. In each case the 5 mg of GABA delayed the onset of effects seen with tubocurarine. It also prevented the preconvulsive phase and convulsions in animals which had received no drug or GABA the week before. Prior administration of a 10 mg dose of GABA gave the same results. It was necessary to give a 15 mg dose of GABA 10 minutes prior to tubocurarine administration to abolish all signs caused by the latter drug.

Discussion. The loud calling by the cat following intraventricular injection of tubocurarine was more prominent and consistent in the present experiments than those of Feldberg and Sherwood(4). With doses of 5 or 10 mg GABA the onset of the calling was always delayed, but never abolished.

The marked sedation and catatonia following GABA administration are consistent with the concept of GABA having a depressant role in the CNS. However, Feldberg and Sherwood

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TABLE I. Summary of Effects of Intraventricular Injection of Tubocurarine and GABA in Cats.

Exp	Drug administered	Drug treatment of previous week	Behavioral effects
1	Tubocurarine*	—	Loud calling, urination, defecation, panting, salivation, mydriasis, fine tremors, increased motor activity, preconvulsive phase of twitching of facial muscles, salivation, collection of froth around the mouth, snapping shut of the jaws and finally clonic-tonic convulsions.
2	"	GABA† or GABA + tubocurarine	As in 1 above but with no preconvulsive phase or convulsions.
3	GABA	Nothing; tubocurarine; or tubocurarine + GABA	Sedation, apathy, hypotonia, scratching movements, catatonia, defecation, urination and lachrymation.
4	GABA + tubocurarine	—	As in 1 above with the effects of tubocurarine delayed and attenuated; the preconvulsive phase and convulsions absent.
5	*Idem*	Tubocurarine	As in 1 above with onset of effects delayed.
6	"	GABA	As in 4 above with less frequent restlessness and more frequent scratching movements.

* All doses of tubocurarine were 40 μg.

† All doses of GABA were 5 mg.

(4) showed that many other pharmacologically unrelated substances also have a CNS depressant effect when injected intraventricularly in cats. Therefore, a strict pharmacological specificity cannot be assigned to this response.

Behavioral responses of the cats injected with a combination of GABA and tubocurarine varied in different experiments depending upon the drug which had been administered the week before. In all of the experiments GABA delayed the onset of the signs caused by tubocurarine and attenuated its effects, *i.e.*, GABA blocked the convulsive seizures produced by tubocurarine. This observation is consistent with that of other workers(8,9) who have reported the inhibitory effect of GABA on other types of experimentally-induced seizures. The drug combination given to a cat which had received an injection of tubocurarine a week before resulted in seizures. Also, tubocurarine failed to cause convulsions in cats which had received an intraventricular injection of GABA the week before. These observations may be due to the effect of GABA being dependent on its intracellular level in the brain and less on the presence of GABA in the CSF(1). However, GABA did antagonize, to some extent, the effect of tubocurarine when it delayed the onset of, and attenuated, the signs following intraventricular tubocurarine.

As reported previously antagonism between GABA and tubocurarine is short-lasting and reversible(10). This has been found also to be the case in the present studies. This transient effect of GABA may be ascribed to its rapid metabolism in the brain or to its inability to build and maintain effective intracellular concentrations during the period when both GABA and tubocurarine are in the ventricles.

It has been shown that some convulsants cause a reduction in the brain content of GABA(2,11). Administration of agents which increase the content of brain GABA protect the animals from experimentally-induced seizures(3). Hence, it is possible that tubocurarine-induced convulsions also cause a diminution in the brain GABA content and that this renders the animal more susceptible to seizures. This may be the reason why cats which had been treated with tubocurarine the week before developed convulsions when administered the GABA-tubocurarine combination, whereas those animals which had received GABA in the first week did not exhibit convulsions when administered the combination or the tubocurarine alone the second week. Presumably, in the cat previously exposed to the tubocurarine the concentration of GABA in the brain was decreased, and it may have been increased in the animals which had received the GABA the week before.

These results indicate that the acute effect of GABA administered intraventricularly in cats is to cause sedation and catatonia, and that there is also a longer-lasting anticonvulsive effect which had been demonstrated in these experiments.

Summary. 1. The effects of intraventricular injections of tubocurarine, GABA and the interaction of these 2 drugs on the behavior of cats had been studied. 2. GABA delayed the onset and attenuated the effect of intraventricularly-injected tubocurarine. The ultimate effect of the combination of GABA and tubocurarine depended upon the drug which had been administered the week before. If it was GABA, no convulsions occurred. If it was tubocurarine, convulsions occurred only after a delay.

1. Elliott, K. A. C., Brit. Med. Bull., 1965, v21, 70.
2. Killam, K. F., Bain, J. A., J. Pharmacol., 1957, v119, 255.
3. Eidelberg, E., Baxter, C. S., Rabels, E., Saldias, C. A., Inhibition in the Nervous System and Gamma Aminobutyric Acid, Pergamon Press, New York, 1960.
4. Feldberg, W., Sherwood, S. L., J. Physiol., 1954, v123, 148.
5. Feldberg, W., Sherwood, S. L., ibid., 1953, v120, 3.
6. Merlis, J. K., Am. J. Physiol., 1940, v131, 67.
7. John, E. R., Killam, K. R., Wenzel, B. M., Tschirgi, R. D., Inhibition in the Nervous System and Gamma Aminobutyric Acid, Pergamon Press, New York, 1960.
8. Gammon, G. D., Gunmit, R., Kamrin, R. P., Kamrin, A., ibid., 1960.
9. Purpura, D. P., Girado, M., Smith, T. J., Gomez, J. A., Electroenceph. Clin. Neurophysiol., 1958, v10, 677.
10. Trivedi, C. P., Domer, F. R., Fed. Proc., 1965, v24, 516.
11. Purpura, D. P., Berl, S., Gonzalez-Monteagudo, O., Wyatt, A., Inhibition in the Nervous System and Gamma Aminobutyric Acid, Pergamon Press, New York, 1960.

Received January 7, 1966. P.S.E.B.M., 1966, v122.

Influence of Restricted Food Intake on Cardiac Glycogen Mobility.* (31076)

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It has been known for some time that fasted animals produce elevated glycogen concentrations in the heart(5). More recently Adrouny and Russell demonstrated that when glycogen was separated into trichloroacetic acid-soluble (TCA) and residual fractions, the greatest change in the fasting animal occurred in the TCA fraction(1). These fractions have been so named because of the method used to isolate glycogen from the tissue. The tissue is homogenized in a trichloroacetic acid medium and then centrifuged. The glycogen in the resulting supernatant is called TCA glycogen and is assumed to be originally free from protein affiliation. The glycogen remaining in the protein residue is called residual glycogen and is thought to be bound to protein.

* This investigation was supported by USPHS Research Grant HE 06747.

Previous investigation has revealed that fasted and nonfasted animals display different degrees of glycogenolysis in TCA and residual fractions when forced to exercise by swimming (3,4). Experimentation in our laboratory has demonstrated that diet as well as fasting influences the glycogen levels of the heart (unpublished results). In the present experiment cardiac glycogen fractions were studied not only in fasted and nonfasted rats with a history of *ad libitum* feeding, but also in animals whose intake had been restricted so that their weights remained constant for 6 months. Animals with these dietary experiences were exercised by swimming so that cardiac glycogen mobility could be studied.

Methods and materials. The cardiac glycogen fractions (TCA and residual) were studied in 2 groups of animals: those on an *ad libitum* diet of Purina Laboratory Chow