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SHORT COMMUNICATIONS

Effect of intraventricular injection of N-methylated GABA-derivatives on the central nervous system of conscious mice

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FOR several years, pharmacological studies have been carried out on the behaviour patterns induced by GABA and related compounds. Recently, their effect in preventing convulsions[1] during electric shock in mice was studied and their activities were tested using isolated hind-gut of crayfish according to Florey's method.

From the above experiments, we speculated on the chemical structure–activity relationship of GABA activity. It may be necessary to have three or four aliphatic carbon chains between the anionic and cationic sites and also to have an anionic charge for the excitable feature, to have a cationic charge for development of depressive symptoms. Furthermore, the relative potency of the anionic and cationic sites, and factors such as the stereo-configuration may be important. The hypothesis we suggest is supported by stretch-receptor experiments in crayfish by McLennan *et al.*[2] and is related to the study of mammalian spinal neurones by Curtis and his co-workers.[3] As a simple method was required, material was injected intraventricularly through the skull into anaesthetized mice, according to the method of Haley and McCormick.[4] The materials employed, were dissolved at a concentration of 0·1–200 μg/0·02 ml in physiological saline.

There was only a depressive effect within 1 min in experiments with physiological saline and pH variation had no influence over pH 3·4. The various amino acids and related compounds mentioned below, were tested. The N-methyl derivatives of aspartic and glutamic acids gave striking results:

N-methyl-DL-aspartic acid monohydrate

Immediately, or about 3 sec after the injection of 0·5–10 μg of N-methyl-DL-aspartic acid, mice developed extreme running convulsions and several minutes later, these changed to typical tonic extensor convulsions and animals died 6–13 sec after the injection. The minimum lethal dose was less than 0·5 μg per animal:

N-methyl-D-aspartic acid

The minimum lethal dose of the D-form was 0·1 μg. The D-form was more active than the DL-form and was the most potent of the compounds tested. The progress of symptoms after injection of the D-form was identical to that of the L-form:

N-methyl-DL-glutamic acid

Running convulsions were evoked 2–3 sec after the administration of 5 μg of this compound, and then symptoms changed through tonic flexor to tonic extensor convulsions before the animals died. After a dose of 50–200 μg of γ-dimethylaminobutyric acid-HCl, a kinetic seizure continued for a few minutes and then the symptoms changed to an excitatory tendency.

With both erythro- and threo-DL-β-oxyglutamic acid, as with N-methylaspartic acid, mice developed running convulsions–tonic flexor–tonic extensor symptoms and the minimum lethal doses of these compounds were all about 10 μg.

For comparison, picrotoxin and pentylenetetrazol were tested. Even 2 min after the injection of 1·5 μg of picrotoxin, clonus had developed in the mice and animals died within 10 min. The action of picrotoxin was as follows: Its latent time until excitement was relatively long, and excitement symptoms became progressively stronger with time till the animals invariably died. Even when 200 μg of pentylenetetrazol were administered, no animals died and its action may be relatively weak. Our

results show that N-methylaspartic acid is one of the most active substances which exists naturally in the brain. It is suggested that N-methylaspartic acid may be an excitatory neurochemical transmitter and that CH_3-group may be important for excitation and depression of brain function.

Details of this work will be published elsewhere in Japanese.

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Uptake of noradrenaline by subcellular particles in homogenates of rat brain*

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THE subcellular localization of catecholamines in particulate fractions prepared from portions of the central nervous system,[1, 2] the adrenal gland,[3–5] and postganglionic sympathetic nerve fibers (splenic nerves)[6–8] has suggested an integral role for the particulate entities in the uptake, storage, and release of these biogenic amines in neural tissue. The object of the present investigation was to determine whether subcellular particulate fractions obtained from the brain of the rat could concentrate and bind exogenous noradrenaline.

Subcellular fractions were prepared at 4° from freshly excised whole brains of decapitated male rats (200 g, Sprague-Dawley). Homogenization was performed for 65 sec in a glass homogenizer (Teflon pestle) containing an aqueous medium of sucrose (0·27 M) and edathamil (Versene, 0·1%); the final ratio of the sucrose medium to brain tissue was 9 to 1. All centrifugations were carried out at 4°. Nuclei and cell debris were removed by centrifugation at 900 × *g* for 10 min. The cloudy supernatant fraction (25-ml aliquot) was decanted and incubated with the priming amine for 30 min at 37°. The incubation mixture was then centrifuged at 100,000 × *g* for 30 min. The high-speed supernatant fraction was decanted and the sediment drained by inversion of the tube, the walls of the tube were wiped free from remaining supernatant fluid, and protein was removed from the sediment by the addition of 0·4 N perchloric acid (6 ml). The extract was passed through a cationic exchange column (Dowex 50-X8),[9] acetate-buffered at pH 6·0, and the catecholamine eluted with 1 N HCl. The noradrenaline content was estimated fluorometrically by a modification of the method of von Euler and Lishajko[10] in which ethylenediamine is added to the ascorbate-sodium hydroxide solution to stabilize the fluorophore.

The noradrenaline content of the final particulate fraction was 61·7 ± 9 (SEM) mμg/g whole brain (Fig. 1, A-1). Incubation of unprimed fractions at 37° for 30 min caused a marked decrease in the level of particulate-bound noradrenaline, whereas the loss occurring after incubation at 4° was minimal (Fig. 1, A-2, A3). The rate of release of endogenous catecholamine from splenic nerve[11] and adrenal medullary[4] granules is similarly temperature-dependent and accelerated at 37°.

The uptake of exogenous catecholamine by subcellular fractions was determined by adding noradrenaline (40 mμg/ml) to the incubation medium at zero time and estimating the noradrenaline

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