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CHARACTER OF CORRELATION BETWEEN THE ANTICONVULSANT ACTION OF PHENAZEPAM AND ITS LEVEL IN THE MOUSE BRAIN

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KEY WORDS: phenazepam; concentration in the brain; antagonism with metrazol.

Inhibition of the convulsant action of metrazol by injection of 1,4-benzodiazepine derivatives into experimental animals is a sensitive method which can be used for screening tranquilizers. By recording minimal effective doses of metrazol causing clonico-tonic convulsions and tonic extension against the background of injection of benzodiazepines it is possible to study quantitatively, in both alternative and graduated forms, the temporal dynamics of the anticonvulsant action of a test compound. In this case it is also possible to study the character of interaction between agonist (metrazol) and antagonist (benzodiazepine) in the formation of the pharmacological response by the agonist.

A method of simultaneous recording of the concentration of phenazepam-^{14}C and its metabolites in the mouse brain and the minimal effective doses of metrazol, intravenous injection of which causes tonic extension in animals, is suggested below. Correlation between these parameters is examined.

EXPERIMENTAL METHOD

Experiments were carried out on female (Black × BALB/c)F_1 mice weighing 18-20 g. Phenazepam-^{14}C (4 Ci/mmole) was injected in Tween emulsion intraperitoneally into the animals in a dose of 1.4 mg/kg (40 μmoles/kg). In the interval from 10 to 120 min after injection of the drug the minimal effective doses of metrazol causing tonic extension were determined in the animals [1]. Immediately after recording of the convulsant action of metrazol the animals were decapitated, the brain (0.36-0.48 g) was removed, and the radioactive material was extracted. For this purpose, the animals' brains were ground with anhydrous Na_2SO_4 (1 : 5 by weight) to form a dry powder, which was extracted with 7 ml chloroform. Model experiments showed that extraction 3 times was sufficient to extract all the radioactive material from the mouse brain. The pooled extracts were then evaporated in a vacuum drying cupboard at 50°C. The dry residue was dissolved in toluene–alcohol scintillator (10 ml) and the quantity of radioactive material was determined on an SL-30 liquid photometer (France). The results were subjected to statistical analysis [2].

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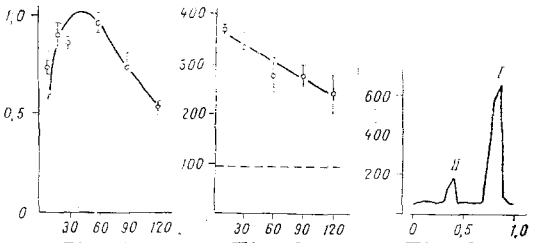

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Concentration of radioactive material in mouse brain. Line shows content of radioactive material calculated by equation (1). Abscissa, time (in min); ordinate, C (in $\mu g/g$ tissue).

Fig. 2. Changes in anticonvulsant action of phenazepam relative to minimal effective doses of metrazol during experiment. Continuous line is regression line corresponding to equation (2); broken line shows control values of minimal effective doses of metrazol. Abscissa, time (in min); ordinate, effective dose (in mg/kg).

Fig. 3. Radiochromatogram of chloroform extract of mouse brain in acetone–chloroform–aqueous ammonia (30 : 70 : 0.5) system. Duration of experiment 1 h. Radioactivity peak (I) corresponds to phenazepam-^{14}C ($R_f = 0.85$), peak II to the 3-hydroxy-metabolite-^{14}C ($R_f = 0.35$). Abscissa, values of electrophoretic mobility (R_f); ordinate, radioactivity (in cpm).

EXPERIMENTAL RESULTS

Determination of the quantity of radioactive material in the mouse brain at different times after injection of phenazepam-^{14}C showed that the process of entry of these substances into and their elimination from an organ obeys a kinetic equation of the first order:

$$C_t = \frac{K \cdot C}{K - \varkappa} (e^{-\varkappa t} - e^{-kt}) \pm \Delta C, \quad (1)$$

where $C = 1.62$ $\mu g/g$ tissue, corresponding to the fictitious dose of the drug entering the brain after injection of phenazepam (1.4 mg/kg) into mice; $k = 2.7$ h^{-1} is the constant of entry; $\varkappa = 0.7$ h^{-1} is the constant of elimination of radioactive material from the animals' brains; t is the duration of the experiment (in h), and $\Delta C = 0.067$ $\mu g/g$ tissue is the error of representativeness of regression at the $P < 0.01$ level.

It follows from kinetic equation (1) that the maximal concentration of phenazepam (1.020 ± 0.067 $\mu g/g$ tissue) was reached in the mouse brain 40 min (0.67 h) after injection of the drug (Fig. 1).

Between the 90th and 120th minutes of the investigation an exponential decline in the content of radioactive material in the brain was observed. During this period there was significant ($P < 0.05$) correlation ($r = +70$) between the coupled values of the minimal effective doses of metrazol giving rise to tonic extension in the mice and the quantity of radioactive material found in the brain. Between the 10th and 60th minutes after injection of the drug, no significant correlation could be found between these indices. Nevertheless, the significant ($P < 0.001$) anticonvulsant effect of intraperitoneal injection of phenazepam-^{14}C into the mice was attained as early as after 10 min and it persisted throughout the period of investigation (Fig. 2). It may be pointed out that the minimal effective dose of metrazol in the control group of animals which gave rise to tonic extension was 94.3 ± 8.4 mg/kg. With time (60-120 min of the experiment), however, the anticonvulsant action of phenazepam declined ($r = -0.59$) significantly ($P < 0.01$); this fall took place strictly ($P < 0.001$) linearly, for the co-

efficient of correlation between the features was 0.62 and the index of linearity of correlation between features (γ) was 0.028 ± 0.070.

The dynamics of the anticonvulsant action of phenazepam can thus be described by the following regression equation:

$$ED\ (\text{in } \mu g/kg) = (a + bt) \pm \Delta ED, \quad (2)$$

where $b = -1.143 \pm 0.316$ is the coefficient of regression (significant at the $P < 0.01$ level); $a = 374.22$ is the free term of regression; $\Delta ED = 32.6$ $\mu g/g$ is the error of representativeness of regression; and t the time of the experiment (in min).

Two other facts deserve attention in connection with the problem under discussion. First, the maximal anticonvulsant action of phenazepam was observed 10 min after its administration (Fig. 2) which did not coincide with the time of the maximum of the content of radioactive material in the brain (Fig. 1). The explanation is evidently the more complex interaction between these two parameters in the early period of investigation. Second, analysis of the radiochromatograms of chloroform extracts of the animals' brain showed (Fig. 3) that they contain two peaks of radioactivity which corresponded to the original preparation (I) and to its 3-hydroxy-derivative (II); their ratio in the brain was 3.5 : 1.

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FURTHER STUDY OF THE ANTIEPILEPTIC PROPERTIES OF NICOTINAMIDE

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KEY WORDS: nicotinamide; strychnine; penicillin; acetylcholine; epileptic activity; complex of epileptic foci; determinant structure.

Nicotinamide is a ligand for benzodiazepine receptors and has an action similar in some of its parameters to that of the benzodiazepines [12-14]. It has been shown that nicotinamide depressed epileptic activity both in a single focus and in a complex of epileptic foci induced in the cerebral cortex with strychnine [7].

The aim of this investigation was to study the antiepileptic action of nicotinamide on foci induced with the aid of substances disturbing different types of inhibition (strychnine, penicillin) or inducing direct depolarization of neurons (acetylcholine—ACH) [9-11, 16].

EXPERIMENTAL METHOD

Acute experiments were carried out on 24 cats. Under ether anesthesia the skin and subcutaneous cellular tissue were divided by a midline incision running from the nasal bones to the occiput. The eye was drained. By trephining the bones of the calvaria and orbit wide access was obtained to different parts of the frontal and temporo-parietal regions of the neocortex. The experiments began 1.5-2 h after administration of ether ceased. The animal was immobilized (tubocurarine 0.1 mg/kg) and artificially ventilated. Scattered

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