DEMONSTRATION OF AN ENDOGENOUS INHIBITOR OF BENZODIAZEPINE RECEPTOR BINDING

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The discovery of receptors (specific binding sites) for various psychotropic drugs in brain tissue has raised the question of the existence of endogenous compounds capable of interacting with these receptors. Attempts have repeatedly been made to isolate such compounds [2, 5]. With respect to drugs of the benzodiazepine series, in particular, several compounds capable of inhibiting binding of a psychotropic drug with its receptor have been described [1, 3, 6, 8], but as yet only those inhibitors which are of peptide nature [3, 6] can be regarded as serious claimants for the role of endogenous ligands of the benzodiazepine receptor.

In this paper an original one-stage method is suggested for obtaining a water-soluble fraction from rat and bovine brain tissue, containing an inhibitor of receptor (specific) binding of benzodiazepines, and some properties of this inhibitor are described. The method is based on a procedure of aqueous extraction of brain tissue at 20°C [4].

EXPERIMENTAL METHOD

A freshly isolated weighed sample of brain tissue was homogenized in 15 volumes (v/w) of distilled water at 20°C and centrifuged at 100,000g and 15°C for 45 min. The supernatant was passed through a filter (Millipore) with pore size of 0.22 μ, after which the ability of the filtrate (hereafter described as extract) to inhibit specific binding of [^{3}H]diazepam (specific activity 94 Ci/mole, from Amersham Corporation, England) with rat brain membranes was studied.

In one series of experiments the extract was concentrated on ultrafilters (from Amicon, Holland) with transmission limits of 500, 2000, 5000, and 10,000 daltons, after which inhibitory activity was determined in filters and concentrates. Specific binding of [^{3}H]diazepam with rat brain membranes was measured as described previously [4, 7] in 1-ml samples in the presence of different quantities of extract. Each sample contained rat brain membranes in a quantity corresponding to 10 mg of the original tissue. The dissociation constant and maximal number of [^{3}H]diazepam binding sites were determined by Scatchard's method.

EXPERIMENTAL RESULTS

The inhibitory activity of extracts obtained from rat brain was 70% greater than activity of extracts from bovine brain.

Maximal inhibitory activity for rat brain was observed in extracts obtained from the cerebellum and cortex, whereas for bovine brain it was in extracts from the thalamus and caudate nucleus.

The use of an ultrafiltration method showed that the molecular weight of the endogenous inhibitor was between 2000 and 10,000 daltons, in agreement with the estimate of 3000 daltons given in [3]. However, in the present experiments partial retention (by 30-40%) of the inhibitor was observed on the filter, with a transmission limit of 10,000 daltons, and this may have been due to its heterogeneity or to absorption on the surface of the filter. The inhibitory activity of the brain extracts was preserved both after heating at 95°C for 15 min and after freezing and thawing (in both cases the decrease in activity was under 5%). Inhibition of specific binding of [^{3}H]diazepam by the endogenous inhibitor resembled in character a dose-dependent effect (Fig. 1). As Fig. 1

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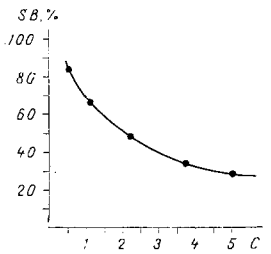

Fig. 1

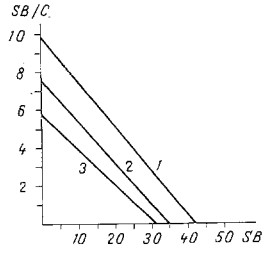

Fig. 2

Fig. 1. Inhibition of specific binding of [^{3}H]diazepam by rat brain extract. Abscissa, concentration (C) in sample (scale unit corresponds to extract isolated from 20 mg whole rat brain); ordinate, specific binding (SB, in percent of control) of [^{3}H]diazepam (9 mM).

Fig. 2. Scatchard plot analysis of specific binding of [^{3}H]-diazepam in the presence of rat brain extract. Abscissa, specific binding (SB) of [^{3}H]diazepam (in pmoles/g tissue); ordinate, ratio of SB of [^{3}H]diazepam (in pmoles/g tissue) to concentration (C) of [^{3}H]diazepam in sample (in mM). 1) Absence of extract, 2, 3) extract isolated from 50 and 100 mg brain tissue respectively added to samples.

shows, 50% inhibition of diazepam binding was achieved by the inhibitor in a concentration ten times less than that in the original tissue.

Determination of the parameters of specific binding showed that in the presence of extract both an increase in the dissociation constant and a decrease in the number of [^{3}H]diazepam binding sites were observed (Fig. 2). Accordingly the data examined above, while affording convincing evidence that the extract contains an inhibitor (or inhibitors) of benzodiazepine receptor binding, do not provide a final answer to the question of the mechanism of its action, for which an independent investigation is required. They can be regarded, in particular, within the framework of the hypothesis of "superhigh" affinity of the endogenous inhibitor for the benzodiazepine receptor. For instance, if the rate of breakdown of the inhibitor–receptor complex is three orders of magnitude less and the corresponding rate for the diazepam–receptor complex, even in the presence of a "purely" competitive mechanism of action of the inhibitor the technique adopted in the present investigation to determine specific binding [7, 8] must reveal the irreversible character of the inhibition.

However, unlike the methods used previously, the proposed one-stage procedure of isolation of extract containing benzodiazepine binding inhibitor is distinguished by its simplicity and reliability; it enables the character of its distribution among different parts of the brain to be evaluated and the extract itself to be obtained in sufficient quantity for further purification and analysis.

LITERATURE CITED

1. C. Braestrup, M. Nielsen, and C. E. Olsen, Proc. Natl. Acad. Sci. USA, Biol. Sci., 77, 2288 (1980).
2. C. Braestrup and M. Nielsen, Trends Pharmacol. Sci., 3, 424 (1980).
3. L. G. Davis and R. K. Cohen, Biochem. Biophys. Res. Commun., 92, 141 (1980).
4. A. Ya. Korneev (Korneyev) and M. I. Faktor (Factor), Eur. J. Pharmacol., 71, 127 (1981).
5. J. L. Martin, Trends Neurosci., 3, 299 (1980).
6. M. Masotti, A. Guidotti, and E. Costa, J. Neurosci., 1, 409 (1981).
7. H. Möhler and T. Ocada, Life Sci., 28, 2101 (1977).
8. P. Skolnik, S. M. Paul, and P. J. Marangos, Fed. Proc., 39, 3050 (1980).