STIMULATION OF SPECIFIC BINDING OF DIAZEPAM WITH MUSCIMOL IN INTENSIVELY WASHED RAT BRAIN MEMBRANES

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The pharmacological action of drugs of the benzodiazepine series is effected through benzodiazepine receptors [2, 10] and is closely linked with the GABA-ergic systems of the brain [1, 5], for GABA and its agonists stimulate receptor binding of benzodiazepines.

Two types of postsynaptic GABA receptors with dissociation constants in the range 10-30 mM (type 1) and 100-350 nM (type 2) for GABA and 2-8 nM (type 1) and 12-70 nM (type 2) for muscimol have now been described [3, 5, 6, 11-13]. Meanwhile the concentrations of semistimulation (EC_{50}) of specific binding of benzodiazepines by GABA and muscimol are 1-2 orders of magnitude higher than their dissociation constants for binding with postsynaptic GABA receptors [4, 7, 8]. On this basis it has been suggested that stimulation of receptor binding of benzodiazepines is effected through a third type of postsynaptic GABA receptors, with very low affinity [7, 8].

The question of the mechanism of stimulation of receptor binding of benzodiazepines by GABA-ergic preparations thus remains unanswered. To elucidate this problem it was decided to study binding of [^{3}H]muscimol and stimulation by muscimol of specific binding of [^{3}H]diazepam on the same preparation of intensively washed rat brain membranes.

EXPERIMENTAL METHOD

The brain of male WAG rats weighing 100-120 g was homogenized in 50 volumes of ice-cold 50 mM Tris-citrate buffer (pH 7.1, 20°C) on a Virtis-45 homogenizer for 30 sec at top speed. The homogenate was centrifuged at 4°C and 20,000g for 30 min. The residue was homogenized in 50 volumes of Tris-citrate buffer and washed by centrifugation, then homogenized a further 12 times in the same volume of buffer. After every four washings the residue was frozen overnight at −20°C. The residue obtained after the last centrifugation was homogenized in 10 volumes of 50 mM Tris-buffer and incubated with 1% Triton X-100 for 40 min at 20°C, and washed twice in 50 volumes of buffer, incubated again in 1% Triton X-100 at 20°C, and washed further twice. The residue obtained after the last centrifugation was homogenized in 50 mM Tris-citrate buffer at the rate of 20 mg of initial weight of tissue to 1 ml of buffer. Samples 1 ml in volume were prepared from the resulting suspension for determination of specific binding of [^{3}H]diazepam (81 Ci/mmole) in a concentration of 0.5 nM, as described previously [4], in the presence of different concentrations of GABA (3 nM-100 μM) or muscimol (1 nM-100 μM). Specific binding of [^{3}H]muscimol (9.5 Ci/mmole, Amersham Corporation, England) was determined in identical samples [12] in a concentration of 6 nM in the presence of various concentrations of muscimol (1 nM-100 μM). To determine dissociation constants specific binding of [^{3}H]muscimol was studied in 12 concentrations between 0.3 and 200 nM, and the results were analyzed by Scatchard's method.

EXPERIMENTAL RESULTS

The study of specific binding of [^{3}H]muscimol with rat brain membranes treated with 1% Triton X-100 revealed two types of binding sites (Fig. 1) with dissociation constants of 1.8 nM (type 1) and 13 nM (type 2) and with concentrations of binding sites of 10 pmoles/g tissue (type 1) and 82 pmoles/g tissue (type 2). Concentrations of binding sites of [^{3}H]muscimol

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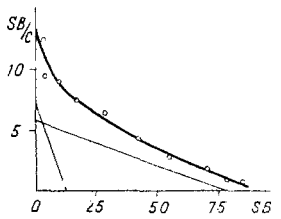

Fig. 1

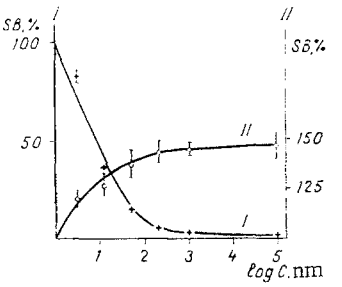

Fig. 2

Fig. 1. Scatchard plot analysis of specific binding of [^{3}H]muscimol with intensively washed rat brain membranes treated with 1% Triton X-100. Specific binding determined in samples of 1 ml containing membrane suspension equivalent to 40 mg of original tissue, in concentrations of [^{3}H]muscimol between 0.3 and 200 nM. Abscissa, specific binding (SB) of [^{3}H]muscimol (in pmoles/g initial tissue); ordinate, ratio between specific binding (SB, in pmoles/g initial tissue) and concentrations (C) of unbound [^{3}H]muscimol (in nM).

Fig. 2. Effect of muscimol on specific binding of [^{3}H]muscimol (6 nM) and [^{3}H]diazepam (0.5 nM) in intensively washed rat brain membranes treated with 1% Triton X-100. Abscissa, logarithm of concentration (log C) of muscimol in sample (in nM); ordinate, % of initial specific binding (SB) of [^{3}H]muscimol (I) and [^{3}H]diazepam (II).

in this investigation were rather lower than those described in the literature [12, 13], evidently because of solubilization of some of the GABA receptors by treatment with 1% Triton X-100 [9, 13].

It will be clear from Fig. 2 that 50% inhibition of specific binding of [^{3}H]muscimol was achieved with a concentration of muscimol of 8.8 ± 0.6 nM. The study of stimulation of specific binding of [^{3}H]diazepam by muscimol yielded a value of EC_{50} of 12.2 ± 2.6 nM (Fig. 2), compared with 180 ± 40 nM for stimulation by GABA (data not shown).

These results show that the concentration giving 50% stimulation of binding of [^{3}H]diazepam by muscimol was very close to the dissociation constant of the type 2 binding sites of [^{3}H]muscimol and to the concentration of muscimol inhibiting binding of [^{3}H]muscimol by 50%. Similar agreement between EC_{50} for binding of [^{3}H]diazepam, obtained in the present investigation, with the dissociation constant of the type 2 binding sites, published in the literature [3, 5, 6, 11], could be observed for GABA.

The concentrations of muscimol and GABA giving 50% stimulation of specific binding of [^{3}H]diazepam obtained in this investigation were lower than those reported by other workers [4, 7, 8]. These differences are probably connected with the more complete removal of endogenous GABA and of other endogenous modulators of benzodiazepine and GABA receptors from the membranes studied [6, 9], which was achieved in the present study by treatment with 1% Triton X-100. Methods of washing used by the authors cited [4, 7, 8] do not guarantee removal of these substances [5, 6, 9], and their presence may have a significant effect on the parameters studied [5, 6].

The results thus show that stimulation of receptor binding of benzodiazepines is evidently mediated through type 2 postsynaptic GABA receptors.

LITERATURE CITED

1. V. V. Zakusov and R. U. Ostrovskaya, Byull. Éksp. Biol. Med., No. 5, 571 (1981).
2. C. Braestrup and R. F. Squires, Proc. Natl. Acad. Sci. USA, 74, 3850 (1977).
3. M. Brovner, F. M. Fercany, and S. J. Enna, J. Neurosci., 1, 514 (1981).
4. T. H. Chiu and H. C. Rosenberg, Eur. J. Pharmacol., 56, 337 (1979).
5. E. Costa, A. Guidotti, and G. Toffano, Br. J. Psychiat., 133, 239 (1978).
6. G. R. Gardner, J. Klein, and J. Grove, Eur. J. Pharmacol., 75, 83 (1982).
7. M. Karobath, P. Placheta, M. Lippitch, et al., Nature, 278, 748 (1976).
8. M. Karobath, P. Placheta, M. Lippitch, et al., in: Receptors for Neurotransmitters and Peptide Hormones, New York (1980), pp. 313-317.

9. M. Mascotti, A. Guidotti, and E. Costa, J. Neurosci., 1, 409 (1981).
10. H. Möhler and T. Okada, Life Sci., 20, 2101 (1977).
11. R. W. Olsen, M. O. Bergman, P. C. Van Ness, et al., Mol. Pharmacol., 19, 217 (1980).
12. N. N. Osborne, Br. J. Pharmacol., 71, 259 (1980).
13. Y. J. Wang, P. Salvaterra, and E. Roberts, Biochem. Pharmacol., 28, 1123 (1979).