or imipramine to potentiate the analgesic action of narcotic analgesics will evidently components for disturbances of systemic regulation arising during the development of pain or of anesthesia. Third, the operation of hypophysectomy, and also administration of the tricyclic antidepressant amitriptyline or the neuroleptic droperidol, which are representatives of different chemical and pharmacological groups, cause hyperalgesia, proof of the neurochemical and morphological polymorphism of this phenomenon and of its predominantly suprahypophyseal etiology.

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ISOLATION FROM BOVINE BRAIN OF SUBSTANCES INHIBITING SPECIFIC BINDING OF IMIPRAMINE AND SEROTONIN UPTAKE

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The discovery of specific binding sites for various psychotropic drugs, including benzodiazepines, neuroleptics, and tricyclic antidepressants, in mammalian brain tissue has raised the question of the possible existence of endogenous ligands for these binding sites. Research in this field has been conducted very intensively [3, 4, 6, 8], due in particular to the role which their detection could play in the task of synthesizing new drugs and in the development of our ideas on brain function under normal and pathological conditions.

The investigation described below is one step in the search for endogenous ligands of the "imipramine receptor" in brain tissue. Since the endogenous ligand interacts in vivo with the same binding sites as the drug, and since one of the important properties of imipramine, on which its pharmacological action is based, is its ability to inhibit serotonin reuptake by nerve endings, in the present investigation an attempt was made to isolate substances from bovine brain tissue capable of inhibiting specific binding of ^{3}H-imipramine and reuptake of ^{3}H-serotonin simultaneously.

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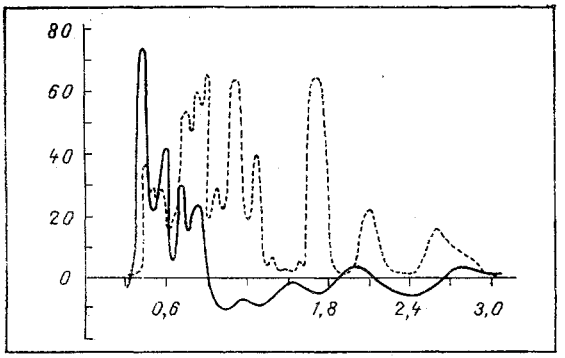

Fig. 1. Typical chromatography of acid extracts of bovine brain tissue on Sephadex G-10. Abscissa, volume of eluate (in liters); ordinate, percentage inhibition of specific binding of ^{3}H-imipramine in presence of corresponding fraction (continuous line) and percentage of absorption at 254 nm (broken line).

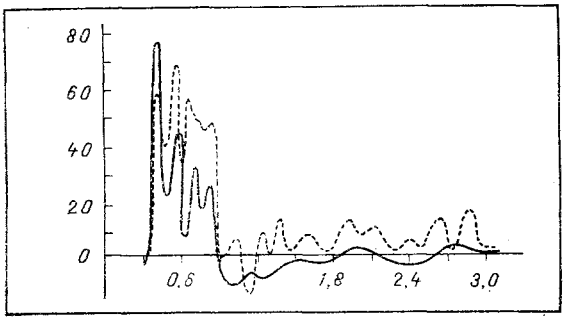

Fig. 2. Profiles of inhibition of specific binding of ^{3}H-imipramine (continuous line) and reuptake of ^{3}H-serotonin (broken line) in presence of fractions of brain extract. Abscissa, volume of eluate (in liters); ordinate, inhibition (in per cent).

EXPERIMENTAL METHOD

Fresh bovine cerebral cortex was homogenized in 0.01 M HCl (1 : 5, w : v) at 80°C for 1 min in an "Ultra-Turrex" homogenizer (IKA, West Germany) at 10,000 rpm. Extraction was carried out at the same temperature for 45 min with constant mixing of the homogenate. The cooled homogenate was then centrifuged for 30 min at 6000 g; the supernatant was centrifuged in an ICF-I continuous-flow rotor (Beckman, USA) at 19,000 rpm, with a flow rate of 60 ml/min. The supernatant was subjected to ultrafiltration, freeze-drying, and chromatography on a column with Sephadex G-10 as described previously [1], the only difference being that deionized water was used as eluent and the rate of elution was 50 ml/h.

Binding of ^{3}H-imipramine with the fraction of unpurified bovine brain synaptic membranes was carried out by the method of Raisman et al. [11]. The incubation mixture contained: 0.1 ml of ^{3}H-imipramine (21 mCi/mmole, from Amersham Corporation, England), final concentration 2 mM; 0.2 ml of the suspension of synaptic membranes, and 0.3 ml of the sample for testing. The bound ligand was separated by vacuum filtration through GF/A filters. Nonspecific binding was estimated in the presence of 10 μM imipramine. The percentage of inhibition (I) was determined by the equation:

$$I = \left(1 - \frac{SB_i}{SB_0}\right) \cdot 100\,\%,$$

where SB_1 denotes specific binding in the presence of the experimental sample and SB_0 binding in the control (deionized water).

Uptake of ^{3}H-serotonin by synaptosomes of rat cerebral cortex was estimated by the method fully described previously [2]. Synaptosomes were incubated at 37°C for 10 min in medium containing 0.45 ml of the test fraction, 0.05 ml of ten times concentrated Krebs–Ringer solution, and 0.5 ml of the suspension of synaptosomes (final protein concentration 1 mg/ml). ^{3}H-Serotonin (specific radioactivity 13.2 mCi/mmole, from Amersham Corporation, England was added in a volume of 25 μl to a final concentration of 20 mM.

EXPERIMENTAL RESULTS

Several fractions containing substances inhibiting specific binding of ^{3}H-imipramine with bovine (rat) cerebral cortical membranes in vitro were isolated by gel-chromatography on Sephadex G-10 from an acid extract of bovine brain tissue. As Fig. 1 shows, most of the substances inhibiting specific binding of ^{3}H-imipramine were contained in the initial fractions of the eluate, corresponding to the fractionation volume under the conditions of chromatography used (from 350 to 900 ml).

Considering the conditions of ultrafiltration of the acid extract of the tissue before chromatography, it can be concluded that all substances applied to the column had a molecular weight of under 5 kilodaltons. It was impossible to estimate the molecular weight of the eluted substances (except the first peak) more accurately, because under the conditions of chromatography used considerable adsorption of material on the matrix of the gel was observed, as shown by the presence of many peaks of absorption at 254 nm in the eluate after the end of the fractionation volume (Fig. 1). Since the first peak of substances inhibiting specific binding of ^{3}H-imipramine came out on the boundary between the dead volume and the fractionation volume, it can be tentatively suggested that the material of this peak could not enter the gel matrix and, consequently, its molecular weight (because of the characteristics of the gel) exceeded 700 daltons, and it thus lay between 700 and 5000 daltons.

The opinion is held [6, 10, 12] that interaction of imipramine with its own binding site is an essential stage in the action of imipramine on serotonin reuptake – one of the important properties which evidently lies at the basis of the pharmacological action of this drug [5, 9].

Investigation of fractions of bovine brain extract with respect to their effect on reuptake of ^{3}H-serotonin by synaptosomes of rat cerebral cortex showed that substances inhibiting reuptake also are contained in the initial fragments of the eluate (Fig. 2). Furthermore, as is clear from Fig. 2, profiles of inhibition of specific binding of ^{3}H-imipramine and of reuptake of ^{3}H-serotonin show sufficiently specific binding of ^{3}H-imipramine and of reuptake of ^{3}H-serotonin show sufficiently close agreement. This suggests that these fractions contain substances capable of inhibiting both imipramine binding and serotonin reuptake by nerve endings. These properties are characteristic of the hypothetical endogenous ligands of the "imipramine receptor," which is manifested at the biochemical level as an imipramine agonist.

The results do not give a final answer to the question of the existence of an endogenous ligand of the "imipramine receptor," but they can serve as the basis for further research, aimed at purifying the active fractions already obtained and identifying the compounds contained in them.

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