CHEMICAL NATURE OF A CONDITIONED TASTE AVERSION FACTOR

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An urgent problem in the physiology of higher nervous activity, which has so far received very little study, is that of the mechanisms of formation of near-natural conditioned reflexes and the phenomenon of their direct "transfer" to untrained animals. It was shown previously [1] for the first time that direct neurohumoral "transfer" of conditioned-reflex taste aversion (CRTA) is possible in rats. "Transfer" of CRTA from trained donor animals to intact recipients by means of brain extracts has been demonstrated on a similar model [7, 8]. The discovery of two types of transfer makes CRTA a promising model with which to study mechanisms of formation of new skills in mammals.

The object of the present investigation was to determine the nature and some of the physicochemical properties of the CRTA for saccharine factor, isolated from brain tissue of trained donors.

EXPERIMENTAL METHOD

Experiments were carried out on male noninbred albino rats weighing 180-200 g. Aversion for saccharine was formed and the recipients selected by methods described previously [1, 4]. Donor animals, in which aversion for saccharine was formed, were decapitated, the brain was removed intact (without the pituitary gland), and frozen in liquid nitrogen, after which it was kept at −20°C. The brain tissue was sampled on the 9th day, for this period from the time of 100% formation of the aversion reaction in the donors is optimal for skill consolidation [4, 9].

To extract active material from the brain tissue of the trained donor animals, extraction with chloroform–methanol–HCl [6] and also extraction in pyridine acetate (0.15 M, pH 5.1) were used. For this purpose, brains frozen in liquid nitrogen were ground in a porcelain mortar to a powdered state, then transferred to a Potter's homogenizer and homogenized for 3 min, with extracting mixture in a volume of 4 ml per brain. The resulting homogenate was incubated at 5° for 30 min, and then centrifuged at 6000g, also for 30 min. The supernatant was poured off and lyophilized. Gel-filtration of the extracts was carried out on Sephadex G-50 superfine and G-25 superfine in 0.06 M $NaHCO_3$ (pH 7.0) and 0.05 M NaCl (pH 6.8). The extract was eluted on columns, calibrated beforehand by molecular weight, at the rate of 5-6 ml/h·cm^2. To test the activity of CRTA for saccharine factor, the lyophilized brain extracts were dissolved in distilled water and injected (in a dose of 50 µl) into the cisterna magna of recipient rats lightly anesthesized with ether. Enzyme treatment of the extracts was carried out with hydrolytic enzymes: pronase P (from Serva, West Germany), trypsin (from Sigma, USA), chymotrypsin (from NBC, USA), and protease-free ribonuclease A (from Sigma) in 0.15 M NaCl (pH 8.0-8.1). To treat 40 mg of lyophilized extract containing 4 mg protein, we used 0.2 ml pronase, 0.2 ml trypsin treated with 0.12 mg TPCK, 0.2 mg chymotrypsin treated with 0.12 mg TLCK (from Serva), and 0.04 mg ribonuclease A. Hydrolysis was carried out for 5 h in the case of proteolytic enzymes and 1 h in the case of ribonuclease. Protein was measured by Bradford's method [5]. Extracts incubated under the same conditions but without enzymes were used as the control. After incubation, the tested material was separated from enzymes by gel-filtration on Sephadex G-25 superfine and

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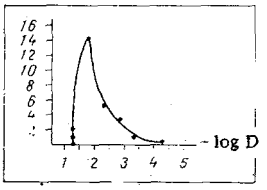

Fig. 1. Effectiveness of transfer of CRTA skill after injection of various doses of brain tissue extract of trained donors. Abscissa, quantity of material injected (doses — D, in equivalents of donor's brain); ordinate, length of time skill persisted in recipient rats (in days).

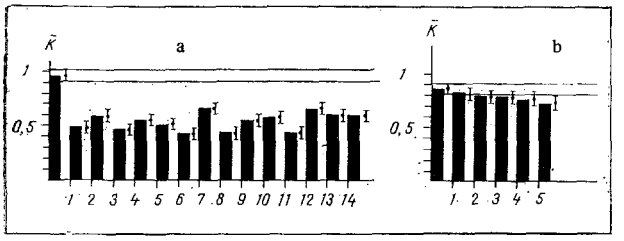

Fig. 2. Drinking behavior of recipient rats after injection of optimal dose (1/40 brain equivalent) of extract of brain tissue of trained donors (A) and of intact animals (B). Abscissa, days of testing; ordinate, mean coefficients of drinking behavior of recipient rats with specified level of significance (0.05).

collected in the elution volume of the active fraction. Control solutions were processed in the same way. To test the temperature-sensitivity of the saccharine aversion factor (SAF) 20 mg of freeze-dried extract in 2 ml of distilled water was boiled for 15 min in a sealed ampul. The residue was then separated by centrifugation at 6000g for 20 min. The resulting supernatant was used for suboccipital injection into recipient rats, which were tested daily for 5 and 14 days, just as during selection. The first testing began 2 h after injection of the extract. For objective evaluation of the drinking behavior of the recipients after injection of extracts of brain tissue from trained donors, a coefficient K was used, equal to the ratio of the quantity (in millimeters) of saccharine consumed by each rat in the course of 15 min to the total volume of liquid consumed:

$$K = \frac{V^{\text{sac}}}{V_{\text{sac + water}}}.$$

A series of experiments was carried out on 9-10 animals and the results subjected to statistical analysis by Student's t test, at a 0.05 level of significance [2].

EXPERIMENTAL RESULTS

After selection of the recipient rats, usually 60% of the total sample of rats remained on the first day, 40-45% on the second day, and 35-40% of the total sample of rats on the third day. To determine the optimal and minimal active doses of brain extracts of trained donor rats (in pyridine acetate), 1/20, 1/40, 1/200, 1/800, 1/2000, and 1/20,000 brain equivalents were injected into intact recipients. The minimal dose of brain tissue extract of aversive donors was found to be 1/2000 brain equivalents, and the optimal dose 1/40 (Fig. 1). The chloroform–methanol extract possessed similar activity in optimal and minimal doses. Investigation of the nature of the SAF thereafter was carried out with the chloroform–methanol–HCl extract.

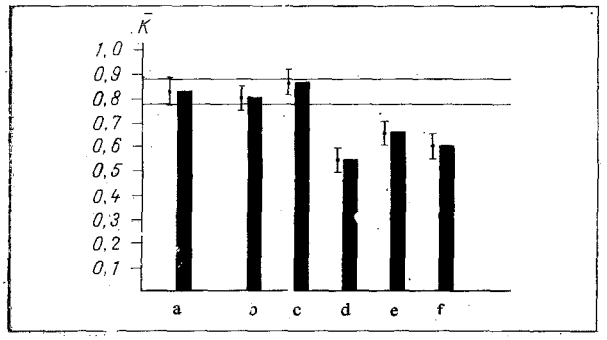

Fig. 3. Action of hydrolytic enzymes on activity of brain tissue extracts from trained donor rats. Abscissa, various types of testing: a) intact recipients before injection of extract, b) brain tissue extract from trained donors, treated with pronase, c) with trypsin, d) with chymotrypsin, e) with ribonuclease A, f) with brain tissue extract from intact animals; ordinate, mean coefficients of drinking behavior of recipient rats during types of testing indicated above.

Aversion for saccharine after a single injection of the optimal dose into selected recipient rats lasted for 14 days. More prolonged observations were not undertaken (Fig. 2a). Injection of brain tissue extracts of untrained rats caused no significant change in drinking behavior (Fig. 2b).

After fractionation of the chloroform–methanol–HCl extract of brain tissue from trained donor animals on a column with Sephadex G-25 activity of the SAF was found in fractions containing substances with molecular weights of under 1200. Treatment of the extracts with pronase and trypsin led to inactivation of the SAF, for no significant change was observed in the consumption of saccharine and water by the recipients. Treatment with chymotrypsin and protease-free ribonuclease A did not inactivate this factor: a significant decrease in saccharine consumption by the recipients took place (Fig. 3). Loss of physiological activity of the low-molecular-weight component of the extract on treatment with proteolytic enzymes demonstrates the oligopeptide nature of the SAF. A significant decrease in the saccharine consumption by the recipients also took place after they had received an injection of the boiled extract, evidence that this factor is thermostable.

The data on the nature and mechanism of action of the SAF agree with Ungar's hypothesis [3] that specific neuropeptides play an important role in the formation of long-term memory during learning.

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