EFFECT OF ANTIBODIES TO DOPAMINE-β-MONOOXYGENASE ON BRAIN CATECHOLAMINE LEVELS

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One of the principal factors inducing schizophrenia and various forms of psychoses is a sharp increase in the dopamine (DA) concentration in presynaptic brain endings, due to a disturbance of the balance between noradrenalin (NA) and DA in the CNS in favor of the latter [5, 10-12]. Biosynthesis of NA from DA is catalyzed by the enzyme dopamine-β-monooxygenase (DBM) (EC 1.14.17.1), which is localized in the brain in neurosecretory granules of adrenergic nerve endings; in response to stimulation of secretion of DBM together with catecholamines through the lymph, they are released into the cerebrospinal fluid and blood [1]. Recently extensive clinical observations have been made [5, 6, 13, 14], demonstrating a significant lowering of the blood and CSF levels of DMB activity in this pathology, which is regarded as one possible cause of the excessive DA formation. Unfortunately, no direct experimental proof of elevation of the DA level in the CNS as the result of depression of DBM activity has yet been obtained.

In the investigation described below we were able to produce the imbalance observed between NA and DA in schizophrenia by blocking DMB by specific antibodies (AB) to the enzyme.

EXPERIMENTAL METHOD

Male albino rats weighing 150-200 g and chinchilla rabbits weighing 1.5-2 kg were used. Electrophoretically homogeneous preparations of DNB were obtained from the adrenal medulla by the method in [7]. Rabbits were immunized with bovine DBM in two stages (at intervals of 30 days) by injection of a solution of the enzyme in 20 mM potassium-phosphate buffer, pH 6.5 (4 mg in 1 ml), in a dose of 0.8 mg protein/kg body weight, into the popliteal lymph node. An electrophoretically homogeneous fraction of AB to DBM (IgG fraction, 150 kD) was obtained from rabbit antiserum by precipitation of the proteins with ammonium sulfate (40%), ion-exchange chromatography on DEAE cellulose (DE-32, from "Sigma," USA), and gel-filtration on sepharose 6B ("Pharmacia," Sweden). The specificity of the rabbit antiserum and of fractions containing AB to DBM was determined by double immunodiffusion on agar ("Difco," USA). The protein concentration was determined by Lowry's method, using bovine serum albumin as the standard [9]. Concentrated preparations of DBM and AB were subjected to dry dialysis using polyethylene-glycol with mol. wt. of 35 kD ("Fluka," Switzerland). A solution of AB (2 mg in 1 ml) in 10 mM sodium-phosphate buffer, pH 7.5, was injected suboccipitally into the rats in a dose of 2.6 μg AB/g body weight. It was shown previously that AB to bovine DBM are cross-reactive against the enzyme isolated from rat brain [15]. The rats were killed 1 h after injection of AB Control animals received either the buffer without AB, or AB inactivated by heat (60°C, 20 min); it was shown beforehand that AB treated in this manner cannot interact with DBM. The concentrations of NA and DA were determined by fluorescence analysis [4], preceded by washing

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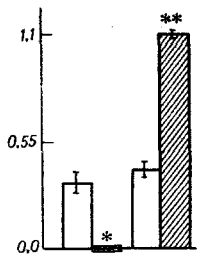

Fig. 1

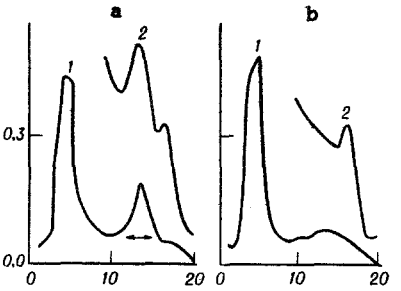

Fig. 2

Fig. 1. Action of AB to DBM on NA and DA levels in rat brain. Ordinate, concentrations of NA and DA (in μg/g brain tissue). *$p < 0.001$, **$p < 0.01$.

Fig. 2. Elution profiles of protein extracts of brain of rats receiving injection of PP-labeled AB (a) and not receiving injection of control AB (b), during gel-filtration on column with sepharose 6B. Ordinate, optical density and relative intensity of fluorescence at 334 nm (1) and 405 nm (2) (λ_{exc} = 334 nm); abscissa, Nos. of fractions. Volume of each fraction 3 ml, zero volume of column, determined from elution volume of blue dextran (2000 kD), 26 ml. Arrows indicate fractions containing PP-labeled AB.

the brain free from blood with physiological saline. Pyridoxal phosphate-labeled AB (covalent cross-linkage relative to ε-amino groups of lysine residues) were prepared as follows: AB (4 mg in 1 ml) were incubated with a 10 times molar excess of pyridoxal phosphate (PP) at pH 8.5 in 10 mM Na_2HPO_4 for 1 h in darkness, after which a saturated solution of sodium borhydride was added and the pH of the incubation mixture was adjusted to 7.5 with 1 M NaH_2PO_4. The excess of pyridoxal phosphate was removed by gel filtration on Sephadex G-50 ("Pharmacia," Sweden), equilibrated with 10 mM sodium-phosphate buffer, pH 7.5. Treatment in this way led to loss of about 30% of the AB (through denaturation), which is within the limits of the assumptions of this method. The concentration of PP incorporated into the composition of AB, was calculated from values of the molar extinction coefficient of the band at 334 nm, characteristic of PP ($E_{334\ nm}$ = 9700 $M^{-1} \cdot cm^{-1}$). As a result of the treatment described above, for each AB molecule there were five PP molecules. Optical measurements were carried out on the Specord M-40 spectrophotometer (East Germany), and "Perkin-Elmer MPF-44A" (Sweden) and "Farrand Optical Co." spectrofluorometers, in cuvettes with an optical path 1 cm long.

EXPERIMENTAL RESULTS

It follows from the results in Fig. 1 that 1 h after suboccipital injection of AB to DBM into the rats there was a sharp fall in the NA concentration in the brain tissue, virtually to zero, and a rise of the DA concentration (about threefold) compared with the initial levels of these compounds in the control animals. Experiments with PP-labeled AB to DBM showed that the AB injected suboccipitally into rats reached the brain tissue. In these experiments, 1 h after injection of AB the rats were killed, the brain was washed with physiological saline to remove blood, after which it was homogenized in 10 mM sodium-phosphate buffer, pH 7.5, containing 0.1% Triton X-100 and 1% NaCl (2 ml buffer to 100 mg brain tissue), in a Potter's homogenizer. The homogenate was centrifuged at 15,000g for 30 min and the residue subjected to the above procedure again. The total supernatant was dialyzed against the same buffer without Triton, and then against the same buffer without NaCl. The solution was then concentrated to 3 ml and subjected to gel-filtration on a column with sepharose 6B (1.8 × 25 cm), equilibrated with 10 mM buffer. The optical absorption at 334 nm and the intensity of fluorescence at 405 nm (λ_{exc} = 334 nm), characteristic of PP, was recorded in the fractions eluted from the column. Elution profiles obtained during gel-filtration of brain extracts of the experimental and control animals are illustrated in Fig 2. These data show that 1 h after suboccipital injection, AB to DMB. labeled with PP were found in protein extracts of the rat brain.

The writers showed previously in experiments in vitro, using a suspension of chromaffin cells, that AB to DBM can pass through the plasma membrane and the membrane of neurosecretory granules, and can thus reach the site of the enzyme and block it [2]. Similar results were obtained [3] when synaptosomes from bovine cerebral cortex and rabbit antiserum were used, and also [8], when whole rat brain and AB to bovine DBM were used. It can thus be asserted with a fair degree of confidence, on the basis of our own results and those obtained by other workers, that after suboccipital injection of AB to DBM they pass through the blood-brain barrier into the brain tissue and reach the site of the enzyme.

On the other hand, we ruled out the possibility of a nonspecific action of AB on DA and NA levels in brain tissue, for no differences could be found between the content of these compounds in the control animals, depending on whether they received the buffer without AB, or AB inactivated by heat treatment.

Thus the use of AB to DBM in order to create a model of schizophrenia in animals seems in our view to be very promising.

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