Formation of Deaminated Metabolites of Dopamine in Noradrenaline Neurons

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Summary. The deaminated monoamine metabolites 3-methoxy-4-hydroxyphenylethleneglycol (MOPEG), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined electrochemically following organic solvent extraction and reverse-phase, high performance, liquid chromatography in four regions of the mouse brain. In the noradrenaline (NA)-predominant regions (hemispheres, brain stem), the ratio of the concentrations of DOPAC plus HVA to NA plus dopamine (DA) was approximately the same as in the DA-predominant regions (corpus striatum, limbic system). Yohimbine and reserpine elevated the concentrations of DOPAC and HVA both in the NA- and the DA-predominant regions. The effect of yohimbine was somewhat enhanced by the α2-receptor blocking agent prazosin in the NA-predominant regions. The concentration of MOPEG was increased by yohimbine and decreased by reserpine.

The concentrations of DOPAC and HVA were lowered by clonidine, but not by apomorphine in the NA-predominant regions of reserpine-treated mice. In the DA-predominant regions, apomorphine, but not clonidine, reduced the concentrations of DOPAC and HVA. The effects of clonidine and apomorphine were reversed by yohimbine and haloperidol, respectively.

The results indicate that the concentrations of the acid DA metabolites DOPAC and HVA in the NA-predominant regions reflect the rate of synthesis of DA in the NA neurons.

Key words: Noradrenaline - Dopamine - Synthesis rate - Acid dopamine metabolites - Receptor specificity

Introduction

Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) appear to be the major metabolites of dopamine (DA) in the brain (Rosengren 1960; Sharman 1963; Andén et al. 1963). The concentrations of these DA metabolites are highest in the corpus striatum, the nucleus accumbens and the olfactory tubercle, i.e., in regions where DA is mainly occurring in special DA neurons (Andén et al. 1963; Westerink and Korf 1976). Besides being a neurotransmitter of its own, DA is also a precursor of noradrenaline (NA). Some of this precursor DA might spill over to the monoamine oxidase and be oxidatively deaminated before it is converted to NA. This possibility was studied in the present investigation, both in normal animals and after treatment with yohimbine and reserpine. These agents probably enhance the synthesis of DA in the NA neurons since they increase the accumulation of DOPA in the NA-predominant parts of the brain following inhibition of the DOPA decarboxylase (Andén et al. 1976; Carlsson 1980). In addition to DOPAC and HVA, we have also determined 3-methoxy-4-hydroxyphenylethleneglycol (MOPEG) and 5-hydroxyindoleacetic acid (5-HIAA) which are the major brain metabolites of NA and 5-hydroxytryptamine, respectively. The experiments were performed in mice, since the deaminated monoamine metabolites appear to be unconjugated in this species (Cesur et al. 1974; Warsh et al. 1981a). The deaminated metabolites were determined using sensitive electrochemical detection following organic solvent extraction and reverse-phase, high performance, liquid chromatography.

Materials and Methods

Male mice of the NMRI strain weighing 20–30 g were used. Care was taken to keep the rectal temperature at 37°C by adjusting the environmental temperature. The animals were killed by decapitation. The brain was quickly removed and placed on an ice-cooled Petri dish. Under an operation microscope, the brain was dissected into the corpus striatum, the limbic system, the hemispheres and the brain stem. The corpus striatum consisted of the caudate nucleus-putamen plus the globus pallidus. The limbic system was separated from the hemispheres using the rhinal fissures as landmark. It contained, i.e., the nucleus accumbens, the olfactory tubercle, the bed nucleus of the stria terminalis, the amygdala, the septum, the prepyriform cortex and the entorhinal cortex. The hemispheres were defined as the cerebral cortex lateral and dorsal to the rhinal fissures, and to this piece, the hippocampus and the cerebellum were added. The rest of the brain was called the brain stem and it consisted of the thalamus, the hypothalamus, the mesencephalon, the pons and the medulla oblongata. The brain parts from two mice were pooled and placed in aluminium foil and frozen on dry ice. The tissue was kept in a freezer (−20°C) for up to 2 days.

Using an Ultra-Turrax homogenizer, the tissue was homogenized in 3 ml ice-cold 0.4 N perchloric acid containing 0.15 ml 10% Na2-EDTA (chelator), 0.1 ml 10% cysteine (antioxidant), 50 μl 3,4-dihydroxyhydrocinnamic acid (50 ng, internal standard) and 200 μl vanillic acid (200 ng, internal standard). After centrifugation and filtration, 2 ml of the extract were neutralized to pH 3.0 by means of N NaOH. The extract was kept in a freezer for up to 4 days. After addition of
1.5 g NaCl, the extract was shaken vigorously with 2 ml distilled ethyl acetate and centrifuged. This procedure was carried out three times. The organic phases were combined and evaporated to dryness under vacuum at 40°C. The residue was dissolved in 0.5 ml mobile phase (see below) and 50 μl were injected into the chromatograph.

The chromatograph consisted of a Waters (Milford, MA, USA) M 45 pump, a Waters U6K injector and two stainless steel columns in series (i.d. 0.46 cm, length of each 10 cm) packed with Nucleosil (Macherey-Nagel, Düren, FRG, 10 μm, RP-18). The following mobile phase was used: 0.015 M K2HPO4, 0.035 M citric acid, HzO, 15 mg/l sodium octyl sulphate (counter ion), 10 mg/l Na2-EDTA (chelator) 8 % v/v methanol, pH 3.9. Without the addition of the octyl sulphate, the small amount of 5-hydroxytryptamine extracted with ethyl acetate interfered with the DOPAC in the chromatogram. The mobile phase was filtered under vacuum through a Whatman paper and evaporated to dryness under vacuum at 40°C. The solvent was pumped at a rate of 1 ml/min (500–1000 psi). The determination was carried out electrochemically by means of a thin layer, flow cell (TL-3, BAS, West Lafayette, IN, USA) containing a carbon paste (CPS, BAS) working electrode, an Ag/AgCl reference electrode and an auxiliary electrode. The potential between the working and the reference electrode was set at +0.85 V. The current produced was monitored using a LC-3 amperometric control unit (BAS) and a potentiometric recorder (OmniScribe, Houston Instruments, Austin, TX, USA).

The concentrations were calculated by comparing the peak heights of the compounds and the internal standard in the sample and in an external standard solution. The external standard was prepared in 0.4 N perchloric acid and treated in the same way as the tissue extract. Dihydroxyhydrocinnamic acid was used as internal standard in the calculations of the DOPAC and 5-HIAA concentrations and vanillic acid was used in those of MOPEG and HVA. Two internal standards were used since it was often observed that the peak heights of MOPEG, HVA and vanillic acid decreased during the day whereas those of DOPAC, 5-HIAA and dihydroxyhydrocinnamic acid remained relatively stable. The reason for the variability of the former substances might be that the 3-O-methylated compounds are not so easily oxidized and, thus, may be influenced by a slight change in the sensitivity of the electrode. From the tissue extracts, dihydroxyhydrocinnamic acid and vanillic acid were recovered to almost the same degree as from the external standard.

In one series of experiments, the endogenous concentrations of NA and DA were determined electrochemically following alumina adsorption and reverse-phase, ion-pair, liquid chromatography (Felice et al. 1978; Andén et al. 1982a).

**Drugs.** The following drugs and chemicals were used: apomorphine HCl·1/2 H2O (Sandoz, Basel, Switzerland), clonidine HCI (Boehringer-Ingelheim*, Stockholm, Sweden), haloperidol (Leo*, Helsingborg, Sweden), prazosin HCI (Pfizer*, Näsbypark, Sweden), reserpine (Ciba-Geigy*, Möland, Sweden), yohimbine HCI (Sigma, St. Louis, MO, USA), 3-methoxy-4-hydroxyphenylethylenglycolhemipiperazine (MOPEG; Sigma, St. Louis, MO, USA), 3,4-dihydroxyphenylacetic acid (DOPAC; Fluka, Buchs, Switzerland), 5-hydroxyindoleacetic acid (5-HIAA; EGA-Chemie, Steinheim-Albuch, FRG), 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid, HVA; Sigma, St. Louis, MO, USA) vanillic acid (Sigma, St. Louis, MO, USA) 3,4-dihydroxyhydrocinnamic acid (Aldrich, Beere, Belgium), ethyl acetate (Kebo, Stockholm, Sweden). Haloperidol, prazosin and reserpine were dissolved in a few drops of glacial acetic acid and 5.5% glucose was added to the volume. The other compounds were dissolved in 0.9% NaCl. The standards were calculated as the free compounds. The doses of the drugs below refer to the salts indicated here.

**Results**

There was more NA than DA in the hemispheres and in the brain stem whereas the reverse was true in the limbic system and, particularly, in the corpus striatum (Table 1). Thus, the ratio between NA and the total catecholamines was high in the former two regions and low in the latter two ones.

The normal concentrations of DOPAC, HVA and MOPEG are also presented in Table 1. The ratio of DOPAC plus HVA to DA plus NA was roughly similar in the four brain regions although possibly somewhat lower in the brain stem. In contrast, the ratio of DOPAC plus HVA to only DA varied greatly. The ratio of MOPEG to NA was much higher in the corpus striatum and somewhat lower in the brain stem than in the other two regions.

Yohimbine markedly elevated the concentrations of DOPAC, HVA and MOPEG in the hemispheres and in the brain stem, i.e., in the NA-predominant regions (Table 2). Prazosin increased the concentrations of these metabolites to a much smaller degree and it possibly potentiated the effect of yohimbine, 3 mg/kg.

The concentrations of DOPAC and HVA in the DA-rich regions, i.e., the corpus striatum and the limbic system, were also increased by yohimbine, although to a somewhat smaller percentage than in the NA-predominant regions (Table 2). Prazosin did not change the concentrations of these metabolites in the corpus striatum and in the limbic system, nor did it increase the effect of yohimbine.

Reserpine increased the concentrations of DOPAC and HVA in the NA- as well as in the DA-predominant regions of the mouse brain whereas it decreased the concentration of MOPEG (Table 3). Clonidine reduced the concentrations of DOPAC and HVA in the NA-predominant regions. This effect of clonidine was completely antagonized by yohimbine. Apomorphine did not lower DOPAC and HVA in the NA-predominant regions.

In the DA-predominant brain parts of the reserpine-treated mice, clonidine did not change the concentrations of DOPAC or HVA (Table 3). They were markedly lowered by apomorphine, however. The effects of apomorphine were completely inhibited by haloperidol but were not significantly changed by yohimbine.

The concentration of 5-HIAA was elevated by reserpine in all brain regions but it was not consistently changed by yohimbine, prazosin, clonidine or apomorphine.

**Discussion**

In the untreated mice, the concentration of DOPAC plus HVA did not follow that of DA but roughly followed that of NA plus DA in the various regions. This finding might indicate that DOPAC and HVA can be formed in the NA as well as in the DA neurons during normal conditions. The ratio of the acid DA metabolites to the total catecholamines...