Resting Secretion of Dopamine from the Adrenal Glands of the Cat in vivo

Though small amounts of dopamine have been reported to be present in the adrenals of sheep[1,2], of the ox[3] and dog[4], it has hitherto not been found in blood[5,6]. This paper demonstrates for the first time the occurrence of dopamine in the adrenal glands of the cat and its secretion into the venous blood under in vivo conditions.

The experiments were performed under ether anaesthesis with 2 cats weighing 2.5 and 2.8 kg. Modifying the 'cava-pocket' method of STEWART and ROGOFF[7], a sac from v. cava inf. was prepared into which only venous blood from the adrenals was emptied. A detailed description is given elsewhere[8]. The heparinized animals (1000 IU/kg) were injected i.v. with 50 mc/kg H^3-3, 5-L-tyrosine (30,000 mc/m*M*)[9]. Then all the effluent blood from the adrenals was collected. The collection periods lasted 5 min; 9 samples were taken per animal. The rate of blood flow through the gland ranged from 1.2 ml/min to 2.1 ml/min. The total volume of blood collected in 45 min was 60 ml. In addition and simultaneously, specimens of arterial blood were taken as controls from the aorta abdominalis. The loss of blood was substituted by infusion of an isotonic electrolyte solution (Tutofusin®). 45 min after H^3-tyrosine injection the animals were decapitated. Adrenals and several other organs (liver, heart, spleen, lungs, brain stem, cerebellum and cortex) were removed as quickly as possible. A special part of the acid soluble fractions (non-volatile compounds of the aqueous phase of the acid soluble fraction after extraction with ethyl acetate) of these organs and of the whole 5 min blood samples was prepared as described in an earlier paper in the presence of carrier quantities of aspartic acid, dopamine, epinephrine, glutamic acid, norepinephrine and tyrosine[8]. The isolation of the catecholamines was carried out by high-voltage paper electrophoresis in a pyridine buffer (details see Figure 1). This system separated dopamine from epinephrine-norepinephrine (Figure 1a). The catecholamines were eluated from the electrophoretograms and fractionated in 2 different ways. The catecholamines of one part of the eluate were acetylated and their 3-O, 4-O, N-triacetyl derivatives were separated on paper chromatograms[10] (Figure 1b). The other part of the eluate was subjected to high-voltage paper electrophoresis in a boric acid buffer[8] in order to determine any contamination with 3-O-methylated catecholamines (metanephrine, normetanephrine and 3-O-methyldopamine). These substances are readily separated from the unmethylated amines because of their inability to form a boric acid complex (Figure 1c). The high-voltage paper electrophoretograms and paper chromatograms were cut into 1 cm strips and the radioactivity was measured with a Tri-Carb (Model EX 314) counter[8,11,12]. The total radioactivity of the electrophoretograms was taken as 100%.

Figure 1a presents a high-voltage paper electrophoretogram of the acid soluble fraction of the venous blood of the adrenals. This sample was taken between 30 and 35 min after H^3-tyrosine injection. H^3-dopamine represents 0.15% of the total radioactivity; 0.25% is localized at the epinephrine-norepinephrine spot. The chromatography of the triacetylated catecholamines (Figure 1b) showed decisively that this radioactivity is only caused by H^3-norephinephrine. The ratio H^3-dopamine/H^3-norepinephrine is about 2:3 in the chromatogram and in the electrophoretogram respectively. The electrophoretogram in boric acid shows that there are no radioactive 3-O-methylated catecholamines present (Figure 1c). H^3-

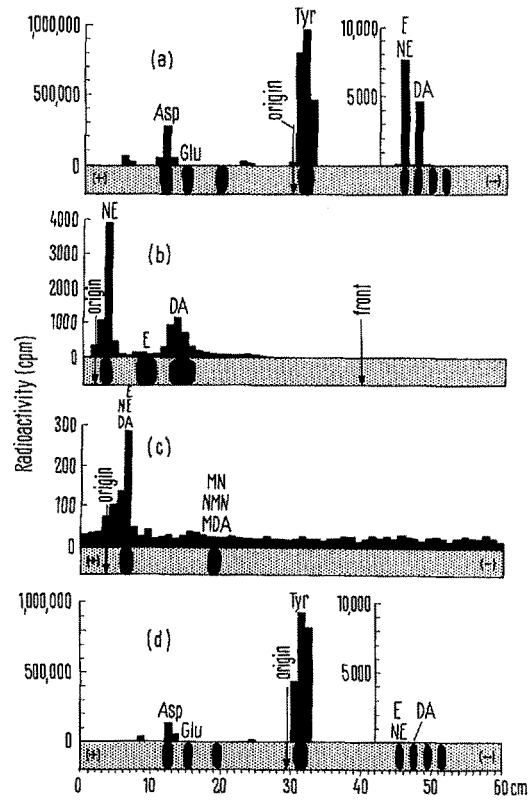

Fig. 1. (a–c) Isolation of H^3-dopamine from the venous blood of the adrenals. The sample was taken between 30 and 35 min after i.v. H^3-tyrosine injection. (d) Control sample of arterial blood from the aorta abdominalis taken during the same time interval. Conditions like (a). (a) Paper high voltage electrophoretogram. Buffer: pyridine/glacial acetic acid/water, 4:1:47 v/v, pH 5,1, field-strength 40 V/cm, $t = -8$ °C, 180 min; paper: Schleicher and Schüll 2043 b Mgl., washed. (b) Paper chromatogram of E, NE and DA, eluated from (a) and transformed to 3-O, 4-O, *N*-triacetylated derivatives. Solvent system: toluene/ethyl acetate/methanol/water, 10:1:5:5 v/v, organic phase[10]. (c) Paper high voltage electrophorectogram of E, NE and DA, eluated from (a). Buffer: boric acid/NaOH/water, 155:16:5000 w/w/v, pH 8.0; 80 V/cm, $t = -8$ °C; 60 min. Abbreviations: Asp, aspartic acid; DA, dopamine; E, epinephrine; Glu, glutamic acid; MDA, 3-O-methyldopamine; MN, metanephrine (3-O-methylepinephrine); NE, norepinephrine; NMN, normetanephrine (3-O-methylnorepinephrine); Tyr, tyrosine.

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catecholamines are not detectable in the arterial blood, as is demonstrated by the electrophoretogram of a corresponding blood sample taken during the same time interval from the aorta abdominalis (Figure 1d).

Figure 2 demonstrates the content of H^3-dopamine and of H^3-norepinephrine in different samples of the venous blood from the adrenals as a function of time after H^3-tyrosine injection. Between 0 and 30 min the concentration of H^3-dopamine and of H^3-norepinephrine increases continuously, and is nearly constant between 30 and 45 min. H^3-norepinephrine concentration reaches values up to $3^0/_{00}$, H^3-dopamine up to $1.8^0/_{00}$ of the total radioactivity. However, in the first sample there is more H^3-dopamine than H^3-norepinephrine, a fact which is easily understood in view of the precursor nature of dopamine.

Besides its occurrence in the venous blood, H^3-dopamine could also be demonstrated in the adrenal gland itself. It represented about 50% of the newly synthesized H^3-catecholamines. Contrary to AXELROD et al.[13], we failed to detect 3-O-methylated catecholamine derivatives in the gland.

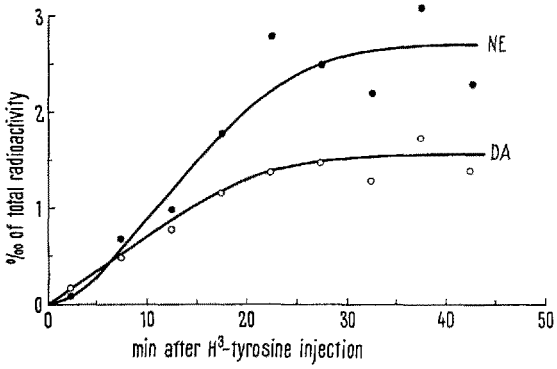

Fig. 2. Concentration of H^3-dopamine (DA) and H^3-norepinephrine (NE) in the venous blood from the adrenals as a function of time after i.v. H^3-tyrosine injection. The total radioactivity of the electrophoretograms was taken as 100%.

Radioactive dopamine was also present in the other organs investigated. The amount of dopamine, expressed as percentage of the total radioactivity of the acid soluble fraction, represents 0.80% in the heart, 0.20% in the spleen, liver and cortex cerebri, 0.17% in the brain stem, and 0.04% in the cerebellum and in the lungs.

The experiments reported here show that dopamine is not only an intermediate in epinephrine biosynthesis in the adrenal gland but also a regular constituent of catecholamines secreted by the adrenal medulla. The biological role of the dopamine secretion cannot be elucidated at this stage. Though dopamine has only little and short lasting pharmacological effect after i.v. administration[14], it must be considered that the fate of secreted dopamine could be influenced by a specific protein binding as suggested recently for catecholamines[15,16].

Zusammenfassung. In Kurzzeitversuchen mit Katzen wurde nach i.v. Injektion von H^3-Tyrosin radioaktives Dopamin im venösen Blut der Nebenniere sowie im Organ selbst nachgewiesen.

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Tumour Glucose-6-Phosphate Dehydrogenase Inhibition by Actinomycin

The pentose cycle, an alternative pathway to the Krebs tricarboxylic acid cycle for aerobic breakdown of glucose-6-phosphate, has been considered by some authors to be more important in neoplastic than in normal tissue[1-5]. The first enzyme participating in this shunt is glucose-6-phosphate dehydrogenase (G-6-PD)[6,7], the activity of which has also been shown by some to be higher in cancer tissues than in their normal counterparts[3,8-10]. Indeed, as suggested by SAHASRABUDHE[5], the high rate of synthesis of nucleic acids in tumours could result in the acceleration of this route for pentose phosphate production. Conversely, inhibition of this cycle or one of its steps might conceivably also inhibit tumour proliferation.

Previous studies using a heterotransplanted human adenocarcinoma (H.Ad.) of probable colonic origin growing in the cheek pouch of unconditioned golden hamsters (*Mesocricetus auratus*), H.Ad. No. 1, showed that tumour-inhibitory doses of actinomycin C resulted in a significant inhibition of tumour G-6-PD activity; corresponding liver G-6-PD activity remained, however, unaffected[11]. In view of the similar chemosensitivity of another heterotransplantable human colonic tumour (GW-39) to H.Ad. No. 1[12,13], it was of interest to determine whether this

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