conclusion of this and other experiments reported recently[6] is that catecholamines stimulate the secretion of luteinizing hormone and inhibit luteotrophin.

Resumen. La administración continuada de α-metil *para*-tirosina, un inhibidor de la síntesis de catecolaminas, impide la hipertrofia compensadora del ovario en ratas. Este efecto es prevenido por la simultánea injección de DOPA, un precursor de las catecolaminas. El grado de la hipertrofia compensadora del ovario no fue modificado por la *p*-cloro-fenilalanina, un inhibidor de la síntesis de serotonina. El estudio histológico de los ovarios de los animales tratados con MPT sugiere que ésta bloquea la ovulación, no modificando aparentemente la secreción tónica de las gonadotrofinas FSH y LH.

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Accumulation of 5-Hydroxydopamine in 5-HT Storage Organelles of Cat Platelets and Concomitant Changes in Platelet Aggregation

Pretreatment of cats with 5-hydroxydopamine (5-HO-DA), an amine acting as a 'false' sympathetic transmitter[1], leads to the accumulation of a highly osmiophilic substance in the vesicles of adrenergic nerve terminals[2] representing 5-HO-DA and possibly its β-hydroxylated and/or *O*-methylated metabolites[1,2]. In blood platelets of different species 5-hydroxytryptamine (5-HT) is mainly localized in specific subcellular organelles and can be visualized by electron microscopy as a highly osmiophilic material[3–5].

In platelets of cats pretreated with 5-HO-DA, the 5-HT storage organelles appeared to contain much more osmiophilic material than under normal conditions. It seemed therefore to be of interest whether 5-HO-DA is accumulated in platelets and whether it displaces 5-HT from the storage organelles as it displaces norepinephrine from the vesicles of adrenergic nerve endings[1,2]. In further experiments we studied whether the accumulation of 5-HO-DA in platelets inhibits their aggregation as is the case after accumulation of 5-HT[6].

Cats of either sex were anaesthetized with 40 mg/kg of sodium pentobarbitone and 20–30 ml of blood were collected from the femoral artery into citrate as described previously[6]. After this first blood collection 20 mg/kg of H^3-5-HO-DA (labelled at the α- and β-C-atom of the side chain, specific activity 0.158 mC/mg) were injected i.v. and further blood samples were collected 30 and 60 min later. Each blood sample was centrifuged with 175 *g* for 10 min at 20–25 °C. The supernatant platelet-rich plasma was used to determine (1) platelet number and platelet volume with a Coulter Counter[7]; (2) the 5-HT content in isolated platelets[8]; (3) platelet aggregation produced by adenosine diphosphate (ADP) and 5-HT measured by a turbidimetric method[9,10]; (4) the radioactivity of 5-HO-DA and its metabolites in isolated platelets (centrifugation of platelet-rich plasma with 3200 *g* for 15 min) and platelet-poor supernatant plasma. After deproteinization with 0.4 *n* $HClO_4$ the radioactivity present in platelets and platelet-poor plasma was separated into acidic and alkaline (amine) fractions on Dowex 50 WX-4 columns. After acetylation the amines were further analyzed by paper chromatography as described previously[1]. In 2 experiments the platelets isolated before and after treatment with 5-HO-DA were prepared for electron microscopy as described previously[3].

Fine structural investigations of blood platelets of 5-HO-DA pretreated animals revealed notable changes in the aspect of the 5-HT organelles. The dense osmiophilic core of these organelles appeared to be on average much larger than those of platelets in control animals. In most instances the dense core now filled the organelle completely, whereas in controls only a partial filling is observed (compare Figure 1 with 2). In addition, after treatment with 5-HO-DA many 5-HT organelles possessed a worm-like, strongly osmiophilic appendix which sometimes surrounded an adjacent clear vacuole (Figures 2b to f). An analogous aspect occurred in control platelets also, although this was exceptional. The number of the 5-HT organelles, counted in ultrathin sections[3] was, if at all, only slightly increased: 116.5 ± 4.8% in platelets of animals 1 h after treatment with 5-HO-DA compared to 100 ± 5.5% in controls. With the exception of the 5-HT organelles no other fine structural changes could be detected in the platelets after 5-HO-DA administration.

Uptake into blood platelets of 5-hydroxydopamine (5-HO-DA) injected i.v. in relation to the 5-hydroxytryptamine (5-HT) content of the platelets

	5-HO-DA μg/10^8 platelets (mean ± S.E.)	5-HT μg/10^8 platelets (mean ± S.E.)
Before 5-HO-DA	–	1.47 ± 0.30
30 min after 5-HO-DA	2.36 ± 0.58	1.31 ± 0.25
60 min after 5-HO-DA	2.02 ± 0.55	1.25 ± 0.22

20 mg/kg 5-hydroxydopamine (1/10 as 3,4,5-Trihydroxyphenethylamine-α,β-$^3H_2$) was injected i.v. into 6 cats after a first blood collection. More blood was collected 30 and 60 min after this injection.

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30 min after the administration of H^3-5-HO-DA, $93.0 \pm 1.5\%$ of the total radioactivity present in platelets consisted of H^3-amines, whereas in the plasma this fraction amounted only to $8.9 \pm 3.0\%$. In contrast to sympathetically innervated organs[1] no β-hydroxylated products of 5-HO-DA could be detected. The relatively small H^3-amine fraction of the plasma consisted mainly of 5-HO-DA and of varying amounts of its *O*-methylated

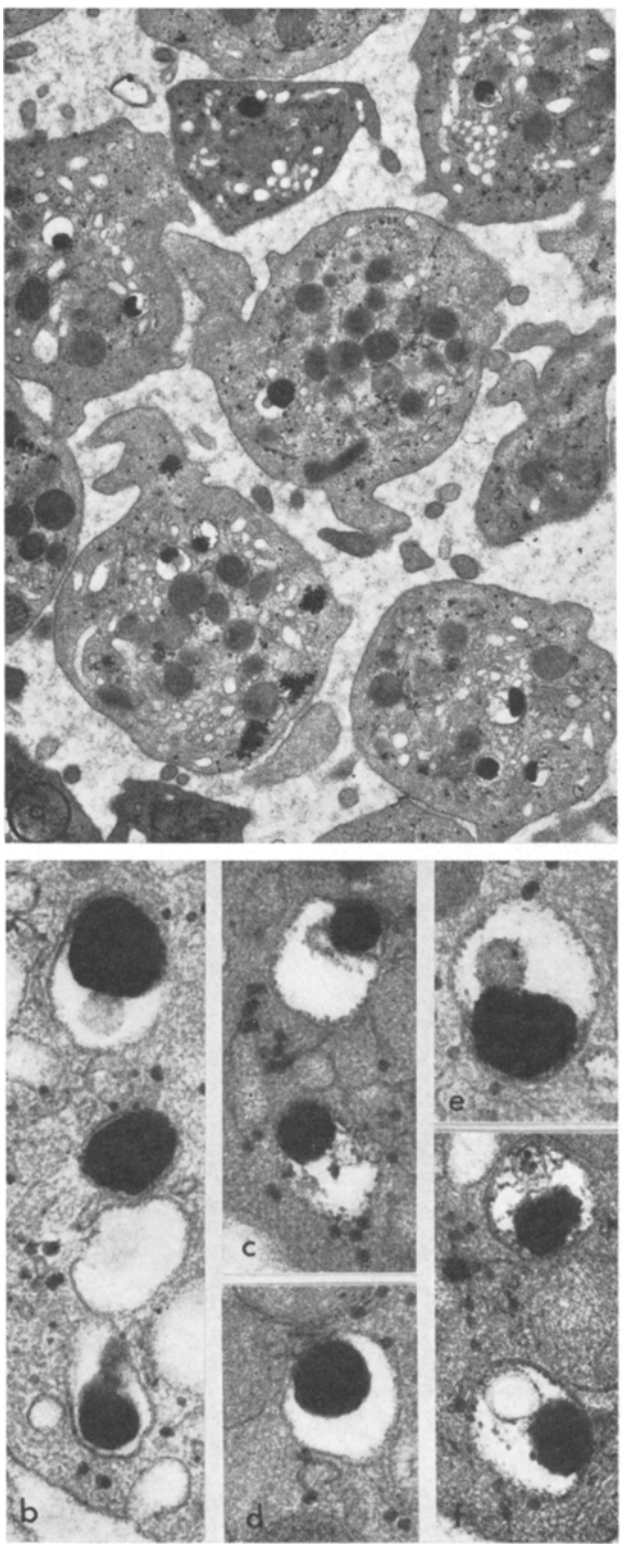

Fig. 1. (a) Electron-micrograph of platelets from an untreated cat. × 14,000; (b) to (f) Some representative aspects of the dense osmiophilic 5-HT storage organelles at higher magnification. × 55,000.

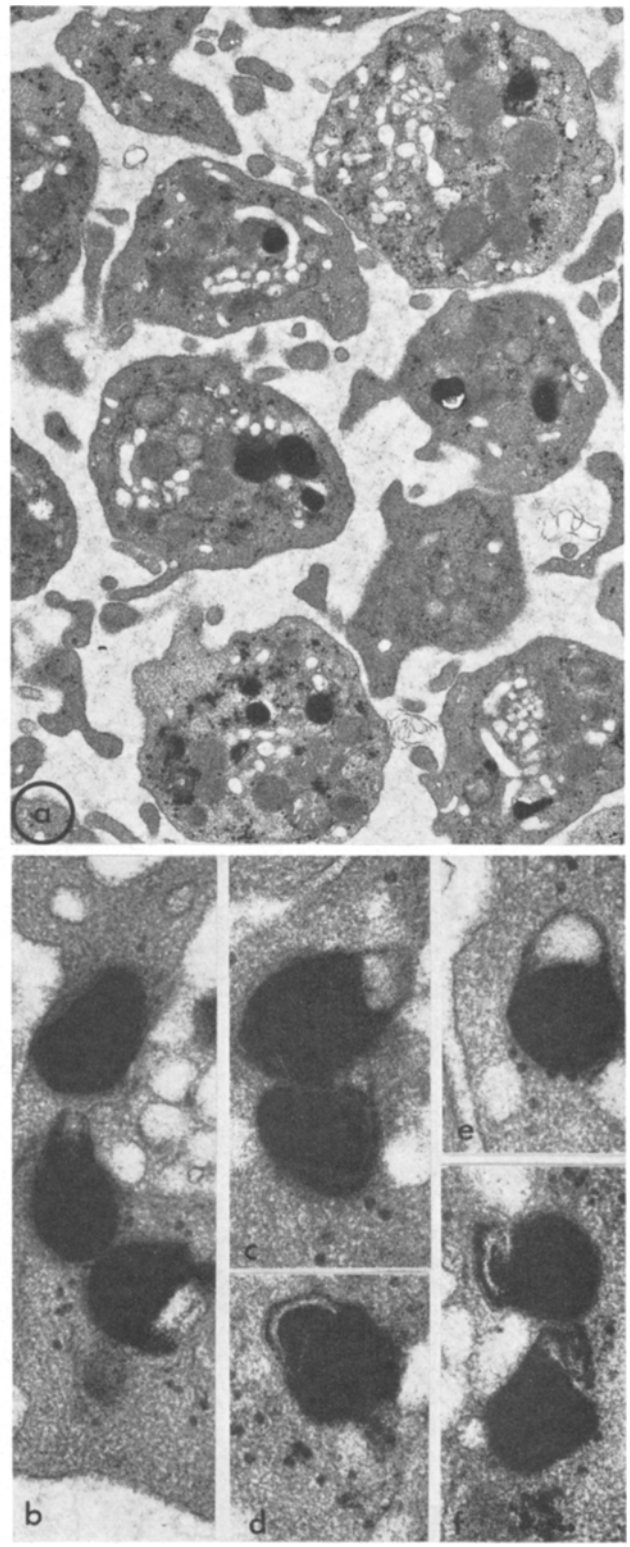

Fig. 2. (a) Electron-micrograph of platelets from the same cat 1 h after the administration of 5-HO-DA. × 14,000. (b) to (f) Various aspects of the 5-HT storage organelles at higher magnification. × 55,000.

or β-hydroxylated metabolites. Both the total radioactivity present in plasma and platelets, and the ratio between H^3-amines and acidic metabolites was virtually the same 30 and 60 min after administration of H^3-5-HO-DA.

30 min after the i.v. injection of H^3-5-HO-DA the content of this amine amounted to 2.36 ± 0.58 $\mu g/10^8$ platelets. This represents a 5-HO-DA concentration in the platelets of 12 mM considering the volume of a platelet of 11.0 ± 1.5 μ^3 before and 11.7 ± 1.3 μ^3 30 min after the injection of 5-HO-DA. Since the concentration of H^3-5-HO-DA in the plasma was only 2.6 μM, the concentration gradient between platelets and plasma amounted to about 5000:1. The calculation of this gradient assumes an equal distribution at 5-HO-DA within the platelets. There is, however, strong evidence that 5-HO-DA is preferentially accumulated in the 5-HT storage organelles as indicated by the marked increase of osmiophilic material after administration of 5-HO-DA (Figure 2).

and after the injection of 5-HO-DA was similar, with the only difference that ADP induced a two-phase aggregation before and a one-phase aggregation after pretreatment with 5-HO-DA.

The 5-HT induced platelet aggregation is possibly mediated by ADP which might be formed by the breakdown of ATP connected to the transfer of 5-HT through the platelet membrane[6,12]. The lack of platelet aggregation by 5-HO-DA added to platelet-rich plasma might be taken to indicate that the transfer of 5-HO-DA through the platelet membrane is not linked to the formation of ADP, i.e. breakdown of ATP.

Nevertheless 5-HO-DA was very efficiently accumulated by the platelets indicating that it has a high affinity to the 5-HT storage sites. The occupation of critically located 5-HT storage sites might prevent a further uptake of 5-HT. Since the uptake of 5-HT is assumed to be linked with the formation of ADP, this would also explain the blockade of the 5-HT induced aggregation.

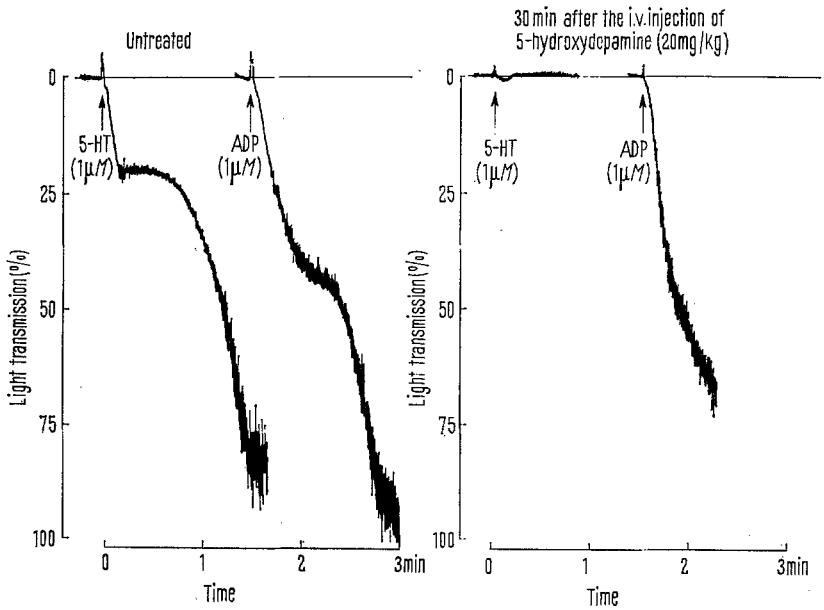

Fig. 3. Platelet aggregation brought about by 5-hydroxytryptamine (5-HT) and adenosinediphosphate (ADP). Before (left) and 30 min after the injection of 5-hydroxydopamine into cats (right). Typical of 10 experiments.

Administration of 20 mg/kg of 5-HO-DA i.v. reduced the norepinephrine content of sympathetically innervated organs of the cat, such as spleen and heart, to about 10%. In contrast the present experiments revealed that the 5-HT content of the platelets was reduced by only 11% and 15% 30 and 60 min respectively after the injection of 5-HO-DA (Table). It seems that the storage capacity of the blood platelets for 5-HT is far from being completely filled up under normal conditions[6]. It therefore seems plausible that 5-HO-DA occupies vacant storage sites without displacing 5-HT.

In previous experiments it has been shown that, in human as well as in rabbit platelets, 5-HT produces a reversible aggregation[7,10,11] irrespective of the dose used. In contrast with platelets of cats, 5-HT in concentrations higher than 1–5 μM always caused irreversible aggregation in 2 phases (Figure 3). Platelets saturated with 5-HT are no longer aggregated by this amine, whether the saturation is effected in vivo or in vitro[6,7]. After pretreatment of cats with 5-HO-DA aggregation by 5-HT was also abolished, whereas the extent and velocity of aggregation brought about by ADP before

Zusammenfassung. 5-HO-DA, das in den Vesikeln der peripheren adrenergen Nervenendigungen gespeichert und als falscher Transmitter freigesetzt wird, wird auch in den Serotonin-Speicher-Granula der Thrombozyten hochgradig angereichert, ohne jedoch das endogene Serotonin freizusetzen. Gleichzeitig wird die durch Serotonin hervorgerufene Thrombozytenaggregation blockiert.

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