Dopamine in the visual cortex of the cat[1]

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Summary. The endogenous content of noradrenaline (NA) and dopamine (DA) was determined by radioenzymatic assays in three different areas of the occipital (visual) cortex, in normal cats as well as in DA-deafferented animals. The use of HPLC methodology enabled us to detect and measure in addition two metabolites of DA: 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), thus confirming the existence of a DA innervation in this cortical region.
Key words. Occipital cortex; dopamine; HVA; DOPAC; radioenzymatic assays.

Extensive neurophysiological, neurochemical and neuropharmacological investigations have supported a role for the catecholamines (CA) in the regulation of cortical neuronal excitability. In the particular case of dopamine (DA), different studies have shown that apomorphine abolishes epileptic photosensitivity not only in experimental models but also in humans suffering from primary or secondary generalized photosensitive epilepsy[3,4]. A simple explanation could be that DA agonists simply block epileptic photosensitivity by inhibiting cortical neurons. In fact, the major effect of DA when applied by microiontophoresis to cortical neurons is to produce long-lasting inhibition in every cortical region examined[5], including the cat visual area[6]. In the past, histofluorescent studies have restricted the cortical DA projections mainly to the frontal cortex[7], and the presence of DA fibers in the occipital cortex has been questioned. Therefore biochemical determinations of DA and noradrenaline (NA) were performed in discrete and well delimited regions of the visual (occipital) cerebral cortex of the cat using a sensitive and specific radioenzymatic assay. The biochemical data was further complemented by the detection and measurement in some of the animals of the major metabolites of DA, thus ruling out the possibility that DA may only be present in the occipital cortex as a precursor of NA.

Methods. This study was performed on 22 adult cats (2.5–3.0 kg) of either sex. In a group of 8 animals, a chemically-induced catecholamine deafferentation was performed by a unilateral diffuse cortical application of 6-OHDA (6-hydroxydopamine hydrobromide; Regis Chemical Co., Illinois) directly applied to the cortical grey matter surface. Briefly, 25-mm diameter circles of Whatman No 2 filter paper were soaked in 1 ml sterile saline with 0.1% ascorbic acid containing 200 µg (free base) of 6-OHDA. About 100 µl of this solution was absorbed on each filter. The dura was opened, but great care was taken not to injure the pia and underlying blood vessels. Two such filters were placed on the cortical surface of each hemisphere for 30 min. Another group of 4 animals underwent diffuse bilateral cortical 6-OHDA applications, using the same procedure. All animals submitted to neurotoxic lesions were pretreated with desipramine (DMI; 20 mg/Kg, i.p.) 30–60 min before the cortical 6-OHDA applications, to block the uptake by NA-fibers. After dural closure and cranioplasty repair, the animals were kept alive for 20–68 days. Finally, a group of four cats were used as controls for the biochemical assays.

The animals were anesthetized (Nembutal, 40 mg/kg; i.p.), and perfused through the aorta with saline. The brains were removed, placed on dry ice and kept frozen at −70 °C until the assays were performed (within 3 days). The brains were gently thawed in the refrigerator (4 °C) and the tissue samples dissected over crushed ice. Great care was taken to remove all visible blood clots, and to remove with a microscalpel as much white matter as possible, so that the samples were composed mainly of grey matter. The samples of occipital cortex included the cortex posterior to frontal (F) = 2 mm, and extending from the midline to lateral (L) = 16 mm[8]. The posterior portion of the intraparietal and the anterior occipital sulcii were used to delimitate laterally the occipital medial cortex, which included part of the lateral and posterolateral gyri. The middle and posterior suprasylvian gyri composed the intermediate occipital cortex, while the lateral occipital cortex included the grey matter lateral to the Sylvian fissure, and therefore was made of the superior temporal gyrus or ectosylvian gyrus[9], and also part of the middle temporal gyrus. The tissue samples were homogenized in 0.1 $HClO_4$, containing 1 mM EGTA and 4 mM Cl_2Mg, the homogenates centrifuged (45 min at 12,500 rpm at 4 °C) and the supernatants decanted for the assays. The pellets were dissolved overnight with 1 N NaOH to measure protein content[10].

The catecholamine (CA) content of the majority of the samples was determined by a radioenzymatic assay based on the methylation of the CA by the enzyme catechol-O-methyltransferase (EC2.1.1.11), purified from rat livers[11], and using [^{3}H]-methyl-S-adenosyl-L-methionine (New England Nuclear or Amersham, 5–15 Ci/mmol) as the methyl donor[12–15]. The [^{3}H]-methylated derivatives were thereafter isolated by thin-layer chromatography[12–16]. In addition, in a group of 6 cats (2 control animals and 4 unilaterally 6-OHDA-treated cats) the assays were performed by high-performance liquid-chromatography (HPLC) with ion-pairing and electrochemical detection[17]. In this series of assays, and since there were no interhemispheric differences in CA values, the control group consisted of 4 samples of cortical tissue from both hemispheres of 2 untreated animals, and 4 samples from the hemispheres contralateral to the treated side of the 4 unilaterally 6-OHDA-treated cats. Briefly, 200 µl aliquots of the $HClO_4$ extract of the tissue samples, filtered over a 0.2-µm mesh (RC-58, Schleicher and Schuell) were injected into a reversed-phase column (5 µm-particles, 250 mm × 4.6 mm Biophase ODS, Bioanalytical Systems; BAS, West Lafayette, IN). The mobile phase was 0.15 M monochloroacetic acid, containing 750 mg/l disodium EDTA, 200–400 mg/l octyl sodium sulphate (Eastman Kodak, Rochester, NY) and 8–10% methanol, adjusted to pH 3.35 with 1 N NaOH, and degassed under vacuum. The column was maintained at 33 °C and the effluent detected at +850 mV using a glassy carbon against the indifferent Ag/AgCl electrode. The flow was kept at 0.8–1.0 ml/min, the different peaks integrated, and their surfaces compared with external standards of known amounts (100 pg-3.0 ng) of NA, adrenaline (AD), DA, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC).

Results and Discussion. In all three regions of the occipital cortex examined, the most abundant catecholamine measured was NA, followed by DA and only traces of AD. The NA content was uniform in three regions (table 1), suggesting a rather homogeneous innervation. In the occipital, medial, intermediate and lateral cortical areas NA represented 57.55%, 67.81% and 71.32% respectively of the total catecholamine content (TCC). After topical applications of 6-OHDA there were slight (−13 to −26%) and non significant reductions in endogenous NA content, due to the blockade of the uptake mechanisms by DMI. Therefore the protection by DMI was considered to be adequate, especially since NA neurons are known to be more sensitive than DA neurons to the neurotoxic effects of 6-OHDA[18]. An interesting aspect was the detection and measurement of AD, since nerve cell bodies, axons and terminals have been described in the brain stem but have not yet been found in the forebrain. The amounts of endogenous AD measured represent a very

Table 1. Endogenous noradrenaline and dopamine content in three areas of the visual cortex in the cat (occipital medial, intermediate and lateral) in controls and in animals treated topically with 6-OHDA

	Noradrenaline ng/mg protein	Dopamine ng/mg protein
Occipital medial		
Controls (n = 16)	2.447 ± 0.215	1.656 ± 0.216
6-OHDA (n = 16)	1.807 ± 0.235	0.900 ± 0.155
Change	− 26.15% NS	− 45.65%*
Occipital intermediate		
Controls (n = 16)	2.549 ± 0.242	1.147 ± 0.160
6-OHDA (n = 16)	2.099 ± 0.256	0.542 ± 0.092
Change	− 17.65% NS	− 52.75%*
Occipital lateral		
Controls (n = 16)	2.318 ± 0.245	0.865 ± 0.087
6-OHDA (n = 16)	2.004 ± 0.298	0.451 ± 0.073
Change	− 13.55% NS	− 47.86% *

Results are expressed as the mean content in ng/mg protein ± standard error of the mean sample. The determinations were performed radioenzymatically. Numbers between brackets represent the number of samples (n). Statistical significance was calculated by unpaired-t-statistical analysis; * < 0.01.

Table 2. Endogenous dopamine, 3-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid; HVA) content in three areas of the visual cortex in the cat (occipital medial, intermediate and lateral) in controls and in animals treated topically with 6-OHDA

	Dopamine ng/mg protein	DOPAC ng/mg protein	HVA ng/mg protein
Occipital medial			
Controls (n = 8)	1.076 ± 0.195	0.337 ± 0.072	1.090 ± 0.451
6-OHDA (n = 4)	0.355 ± 0.056	0.135 ± 0.072	0.117 ± 0.042
Change	− 67.01% ***	− 59.70% *	− 89.27 **
Occipital intermediate			
Controls (n = 8)	0.573 ± 0.108	0.450 ± 0.108	1.236 ± 0.360
6-OHDA (n = 4)	0.202 ± 0.068	0.196 ± 0.079	0.227 ± 0.077
Change	− 64.75% **	− 56.44% *	− 81.63% **
Occipital lateral			
Controls (n = 8)	0.545 ± 0.118	0.250 ± 0.058	0.679 ± 0.248
6-OHDA (n = 4)	0.162 ± 0.055	0.122 ± 0.060	0.267 ± 0.165
Change	− 70.28% **	− 51.20% *	− 60.68% *

Results are expressed as the mean content in ng/mg protein ± standard error of the mean sample. The sample of control cortex (n = 8) are the values obtained from both hemispheres of 2 control cats (4) and from the contralateral hemispheres of the 4 unilaterally-treated cats (4). The assays were performed by high performance liquid chromatography.
Numbers between brackets represent the number of samples (n). Statistical significance was calculated by the nonparametric test of Mann-Whitney.
* $p < 0.05$; ** $p < 0.02$, and *** $p < 0.01$.

small fraction (< 4%) of TCC, and the enzyme phenylethanolamine-N-methyltransferase has an extremely low activity or remains undetected in cortex, so that it is at present impossible to analyze the distribution of AD with biochemical methods alone. Furthermore, since there is a certain overlap (crossover) in the TLC separation of NA and ADR[13,14] further speculations are not at present justified.

The distribution of endogenous DA had a medial to lateral gradient; i.e. it was most abundant in the medial occipital cortex (1.6 ng/mg protein; 38.95% of TCC), followed by the intermediate occipital region (1.1 ng/mg protein; 30.51% of TCC), and smaller amounts were determined in the lateral area (0.865 ng/mg protein; 26.62% of TCC). After the topical 6-OHDA treatment, there were significant reductions in DA, HVA and DOPAC (tables 1 and 2). If the endogenous DA measured in the occipital cortex represented only a precursor of NA, one would expect a uniform distribution, paralleling that of NA, and no changes after a 6-OHDA treatment, which does not affect NA fibers protected by DMI. Finally, the detection and measurement by HPLC of DA and two of its main metabolites; i.e.: DOPAC and HVA, not only rules out artifactual errors of the radioenzymatic assay (separation of NA and DA) but confirms the presence of an authentic DA system projecting to the ocipital cortex in the cat. The average DA levels determined by HPLC were slightly lower than those measured radioenzymatically, but still in the same range of individual values, and also showed a medial to lateral gradient.

The majority of the studies on the monoamine innervation of the neo-cortex have been performed in the rat, so that there is only sparse information concerning other species, such as the cat. For the rat brain, most histofluorescent studies have demonstrated DA nerve fibers as having their terminal fields only in restricted cortical areas, mainly in the frontal pyriform and entorhinal regions[7,19]. Although biochemical studies have shown that DA is found in many regions of rat[16,20,21] and cat[15,22,23] cerebral cortex, its presence when determined biochemically does not warrant the identification of a dopaminergic pathway, since DA it is the natural precursor of NA. Interestingly, after extensive bilateral electrolytic lesions of the locus coeruleus region in the cat there is a significant decrease in NA levels (−85%) but DA content remains unchanged in the occipital cortex[22]. It has also been documented by combining retrograde transport of horseradish peroxidase and CA histofluorescence that there is a DA projection from the ventral mesencephalic tegmentum to the visual cortex in the cat[24].

Because of the difficulties inherent in methods using histofluorescence, and in spite of the fact that several biochemical studies have documented more extensive DA projections than those originally described by fluorescence microscopy for the rat brain, the existence of DA-containing nerve fibers in the occipital region of the cat has often been questioned. It is only recently that new dopaminergic terminal fields have been visualized in the motor, visual and retrosplenial cortex of young and adult rats, using combined histofluorescence and immunocytochemical approaches[25]. The present biochemical survey confirms the existence of DA-containing nerve fibers in the visual (occipital) cortex of the cat by two different assay procedures, including the detection and measurement of the two metabolites DOPAC and HVA. In addition, it points to a differential and heterogeneous distribution, according to the region of the occipital cortex examined. However, a more precise and definite quantification of the DA innervation in the cerebral cortex, including the laminar or in depth intracortical distribution[23], will eventually require radioautographic[26,27] and/or immunocytochemical[28] labeling of presumptive DA fibers and their axonal varicosities.

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Extraneuronal serotonin accumulation in peripheral arteries of the rat

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Summary. Accumulations of serotonin (5-HT) and norepinephrine (NE) were compared in control and 6-hydroxydopamine (6-OHDA) pretreated rat aorta, mesenteric and tail arteries. The distribution of these amines was corrected by subtracting tissue uptake of tritiated sorbitol in the extracellular space. 5-HT greatly accumulated both in control and 6-OHDA pretreated arteries. In contrast, NE accumulation in mesenteric and tail arteries was substantially decreased after 6-OHDA treatment. In the aorta 6-OHDA pretreatment did not affect the accumulation of both amines. These findings suggest that 5-HT accumulation in these arteries is mainly extraneuronal, and NE mainly neuronal. Since the accumulation of 5-HT in the aorta was not influenced by pretreatment with 10 μM NE, the extraneuronal uptake mechanisms for 5-HT and NE appear to be different.
Key words. Serotonin; uptake; vascular smooth muscle; rat.

Among vasoactive substances, serotonin (5-HT) has been considered to be one of the most active vasocontractile substances[1]. Its inactivation such as by uptake into specific sites therefore will play an important role in determining the vascular tone. Unlike norepinephrine (NE) which is primarily taken up into sympathetic neuronal tissues in most vascular beds[2], 5-HT uptake into neuronal[3] and/or extraneuronal tissue[4] remains unclear. We have, therefore, examined and compared the accumulation of 5-HT and NE in several control and sympathetically denervated vascular tissues of the rat.

Materials and methods. Male Sprague-Dawley rats weighing 280–330 g were anesthetized with intraperitonial pentobarbital (50 mg/kg), and heparin (2 mg/kg) was injected via inferior vena cava. The aortic, mesenteric and tail arterial preparations were dissected out and kept in a modified Krebs solution bubbled with 95% O_2 and 5% CO_2. The composition of the Krebs solution at 37 °C was as follows (mM): NaCl 122.0; KCl 5.2; $CaCl_2$ 2.4; $MgSO_4$ 1.2; $NaHCO_3$ 25.6; KH_2PO_4 1.2; disodium EDTA 0.03; ascorbic acid 0.1; dextrose 11.0 (pH 7.4). After removing excess surrounding tissues, the specimen was cut open to remove any blood clots and incubated with 0.1 mM pargyline for 30 min. It was then washed with fresh Krebs solution three times every 10 min, and cut into 4-mm-long segments. The tissues were incubated for 5 min with 2 ml Krebs solution containing 0.1 μM [^{3}H]-5-HT or [^{3}H]-NE (16.9 Ci/mmol and 34.0 Ci/mmol, respectively, New England Nuclear, Boston, MA). After incubation, the tissues were blotted between two pieces of filter paper moistened with two drops of Krebs solution and immediately weighed using a Perkin-Elmer electrobalance (AD-2Z). The tissues were digested with 0.5 ml BTS-450 (Beckman) at 37 °C in closed vials. After addition of 4.0 ml of Ready Solv NA (Beckman) to the vials, radioactivity was measured with Beckman liquid scintillation counter (LS-5800). For chemical denervation using 6-OHDA, the tissues were incubated in Krebs solution containing 0.1 mM 6-OHDA for 30 min followed by incubation in 6-OHDA-free Krebs solution for another h at 37 °C[5,6]. The complete adrenergic denervation was confirmed by the disappearance of catecholamine fluorescence[6]. In [^{3}H]-NE uptake experiments, 0.1 mM U-0512, an inhibitor of catechol-O-methyl transferase, was added to the incubation medium.

For determination of extracellular space, tissues were incubated in Krebs solution (37 °C) containing 0.5 μM [^{3}H]-sorbitol (24.0 Ci/mmol, New England Nuclear, Boston, MA) for 5 min. The tissues were then blotted, weighed and digested as described above. The distribution of 5-HT in extracellular space was corrected by subtracting [^{3}H]-sorbital uptake.

The data were expressed as means ± SEM. For the statistical analysis of the data, Student's t-test for unpaired observation was used. P-values less than 0.05 were considered to be significant.

Results. Results of the present study demonstrate that 5-HT is greatly accumulated in the rat aorta, mesenteric and tail arteries (fig.). The accumulations of 5-HT in 6-OHDA pretreated mesenteric and tail arteries remains greater than 50% of the control arteries. The accumulations of 5-HT in 6-OHDA pretreated mesenteric and tail arteries were 61.7 and 59.8% of their respective control arteries. 6-OHDA pretreatment, however, did not affect the 5-HT accumulation in the aorta. Substantial NE accumulation was also found in control mesenteric and tail arteries. Accumulations of NE in aorta however were little. The accumulations of NE in 6-OHDA treated mesenteric and tail arteries were less than 50% (38.2 and 46.5%, respectively) of those in intact arteries. The accumulation of NE in the aorta was not affected by 6-OHDA pretreatment. The accumulations of 5-HT in aorta and mesenteric artery were greater than those of NE in the respective control and 6-OHDA-pretreated arteries (fig.). The accumulations of 5-HT in control and 6-OHDA-pretreated aortae were not influenced by pretreatment of 10 μM NE.