Dissociation Between Biochemical and Ultrastructural Effects of 6-Hydroxydopamine in Rat Brain

It has been reported that 6-hydroxydopamine (2,4,5-trihydroxyphenylethylamine) induces a long-lasting depletion of norepinephrine from peripheral sympathetically innervated organs[1,2] and that this depletion is a consequence of a selective degeneration of adrenergic nerve terminals[3,4]. Intracerebral injection of 6-hydroxydopamine results in a disappearance of catecholamine fluorescence in brain neurons[5]. Moreover, intraventricular administration of the drug decreases the brain concentration of norepinephrine[6,7] and dopamine[7] and markedly reduces the uptake of tritiated norepinephrine into brain slices[6]. However, the mechanism by which 6-hydroxydopamine induces such a modification in brain amines has not yet been clarified.

The present study shows that, in the brain, 6-hydroxydopamine may decrease the catecholamine content without apparently causing ultrastructural damage.

Methods. Male albino rats of Wistar origin (Füllinsdorf), weighing 250–280 g, were implanted with a permanent cannula into the right lateral ventricle of the brain[8]. 10–15 days later, 200 µg of 6-hydroxydopamine (base, dissolved in 10 µl saline)[9] were injected intraventricularly once or twice with an interval of 24 h. 2 or 5 days after the single or the second administration, the animals were sacrificed for biochemical and ultrastructural investigations. Animals injected with saline served as controls. In the brain (without cerebellum and medulla oblongata), dopamine and norepinephrine[10] (with minor modifications[11]) and serotonin[12] were determined.

For electron microscopical examinations, the brain was fixed by vascular perfusion as previously described[13]. Hypothalamus periventricularis and caudate nucleus were postfixed in osmium tetroxide, dehydrated and embedded in Epon. Ultrathin sections were double-stained with uranyl acetate and lead citrate.

Results. (1) 2 days after a single treatment with 200 µg 6-hydroxydopamine, the cerebral content of both dopamine and norepinephrine was reduced to 68 and 35% of controls respectively, whereas 5-hydroxytryptamine was decreased to 88% only (Table).

(2) Electron microscopical examinations of both hypothalamus periventricularis and nucleus caudatus 2 or 5 days after a single injection of 6-hydroxydopamine revealed no apparent ultrastructural changes of nerve terminals and neuronal perikarya compared to tissues of animals injected with saline (Figure, a). However, the administration of twice 200 µg 6-hydroxydopamine induced ultrastructural alterations in the hypothalamus and the nucleus caudatus. Both areas showed preterminal and terminal neuronal profiles of increased size, a numerical reduction of the small agranular vesicles, lysis of mitochondria, and proliferation of neurofilaments and neurotubules (Figure, b–d). Some preterminal structures were particularly enlarged (Figure, c). Besides, neuronal profiles of normal appearance occurred (Figure, d).

Discussion. The present observations confirm the decrease of brain norepinephrine[6,7] and dopamine[7] after intraventricular injection of 6-hydroxydopamine. Moreover, as receatly shown[14] the brain 5-hydroxytryptamine is less affected than the catecholamines indicating a relatively specific action of 6-hydroxydopamine on catecholaminergic neurons.

In the peripheral sympathetic nervous system, the depletion of norepinephrine by 6-hydroxydopamine has been demonstrated to be related to a degeneration of nerve terminals. Besides, a replacement of norepinephrine by 6-hydroxydopamine (false transmitter action) has also been proposed[14] suggesting that the structural damage may not be the only causative factor of catecholamine depletion. The present experiments in the central nervous system clearly show that 6-hydroxydopamine (200 µg intraventricularly) can deplete catecholamines without causing apparent ultrastructural changes. The decrease of cerebral catecholamines might be due to their replacement by 6-hydroxydopamine (false transmitter action) and/or to inhibition of their synthesis as indicated by preliminary experiments with ^{14}C-tyrosine and ^{14}C-dopa[15].

Repeated doses of 6-hydroxydopamine (2×200 µg) in addition cause structural damage in the caudate nucleus and the hypothalamus (Figure, b–d) but, compared to 200 µg 6-hydroxydopamine given only once, do not markedly enhance the decrease of cerebral norepinephrine[6]. The degenerative features, basically evaluated according to GLEES and HASAN[16], are similar to those occurring in the peripheral sympathetic nervous system after 6-hydroxydopamine[3]. Also the very large preterminal profiles observed (Figure, c) resemble those appearing after nerve section[17] and may be the ultrastructural

Effect of 6-hydroxydopamine (6OHDA) on catecholamines and 5-hydroxytryptamine in the brain of rats

	Controls (µg/g)	6OHDA (µg/g)	6OHDA % of controls
Dopamine	+ 0.85 ± 0.04	0.58 ± 0.10	68.0[a] ± 2.9
Norepinephrine	0.54 ± 0.06	0.19 ± 0.01	35.4[a] ± 3.3
5-Hydroxytryptamine	0.49 ± 0.04	0.43 ± 0.02	88.3[a] ± 3.2

200 µg of 6-hydroxydopamine were injected into the lateral cerebral ventricle 48 h before sacrifice. The values are expressed in µg/g and in % of controls. Means with standard error of 3–4 duplicate determinations each from a pool of 2 rat brains. [a] $p < 0.01$.

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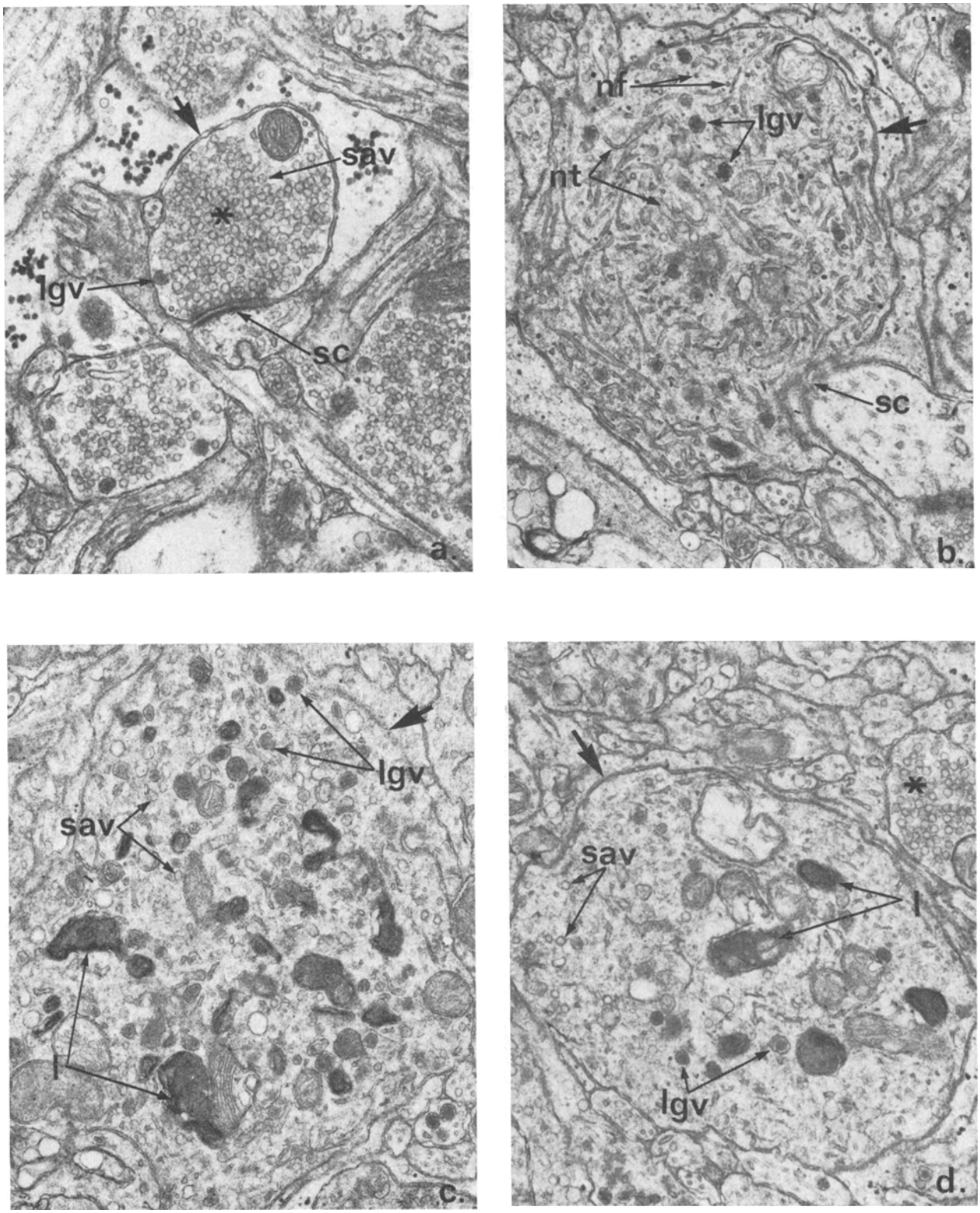

Ultrastructural aspects of rat brain regions 48 h after injection of 6-hydroxydopamine. (a, b, c) hypothalamus periventricularis after 200 µg (a) and 2 × 200 µg (b, c) 6-hydroxydopamine; (d) nucleus caudatus after 2 × 200 µg 6-hydroxydopamine. Large arrow: neuronal cell membrane delineating terminal (a, b) or preterminal (c, d) neuronal profiles. l, lysed mitochondria; lgv, large granular vesicles; nf, neuronal filaments; nt, neuro tubules; sav, small agranular vesicles; sc, synaptic contacts; *, 'normal' nerve terminal. × 31,000.

correlate of the 'strongly fluorescent bulges' described in the periphery[4]. These possibly represent the site of accumulation of storage vesicles where the axon is interrupted.

The dual action of 6-hydroxydopamine remains to be clarified. The compound, owing to its chemical similarity to dopamine, might be transported into the catecholaminergic nerve endings by the same specific mechanism as the endogenous catecholamines. As a consequence, 6-hydroxydopamine would accumulate in the nerve terminals and may replace the endogenous catecholamines and/or inhibit their synthesis (see above). As long as the intraneuronal concentration of 6-hydroxydopamine and/or its potential metabolite 6-hydroxynorepinephrine is relatively low (as after once 200 µg 6-hydroxydopamine), no ultrastructural changes appear. Higher intraneuronal concentrations of 6-hydroxycatecholamines (after twice 200 µg 6-hydroxydopamine) evidently cause in addition morphological damage. This might be due, for instance, to disruption of the tertiary and quarternary structure of proteins in the nerve endings, since 6-hydroxydopamine is a derivative of 6-hydroxyhydroquinone, a strong reducing agent.

In conclusion, 6-hydroxydopamine seems to have two main actions. A single dose decreases the cerebral norepinephrine and dopamine, possibly by acting as a false transmitter and/or by inhibiting catecholamine synthesis. Repeated doses, in addition, cause specific ultrastructural lesions of the catecholaminergic neurons similar to those found in the peripheral sympathetic nervous system[18].

Zusammenfassung. Im Gehirn von Ratten bewirkt einmalige intraventrikuläre Injektion von 200 µg 6-Hydroxydopamin nach 2 und 5 Tagen eine deutliche Verminderung von Noradrenalin und Dopamin, während 5-Hydroxytryptamin nur geringfügig herabgesetzt wird. Elektronenmikroskopisch sind keine strukturellen Veränderungen festzustellen. Zweimalige Injektion von 200 µg 6-Hydroxydopamin führt dagegen zu schweren spezifischen Degenerationen im Bereich des Nucleus caudatus und Hypothalamus. Daraus wird geschlossen, dass niedrige Dosen 6-Hydroxydopamin die cerebralen Catecholamine vermindern können, ohne ultrastrukturelle Schädigungen des Gehirns zu verursachen.

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Carotenoids of *Betaeus harfordi* (Crustacea: Decapoda)[1]

Betaeus harfordi is a small shrimp which lives in the mantle cavity of abalone. It occurs along the coast of California and part of Mexico in all species of *Haliotis* but most commonly in the pink abalone, *Haliotis corrugata*[2].

The present investigation was undertaken for the purpose of characterizing the total carotenoid pigments of *Betaeus harfordi* and to determine the carotenoid moiety of the supposed caroteno-protein which causes the deep blue-purple external body color.

Methods. Animals which had been starved for 5 days were used for the study. The carotenoids were extracted with ethanol and washed thence by dilution into hexane. Absorption spectra were determined on a Bausch and Lomb spectronic 505 recording spectrophotometer. Thin-layer chromatography was conducted upon silica gel H using eluants composed of 15% acetone in hexane (v/v) for zones 1–4 and 25% acetone in hexane for zone 5. The carotenoids were identified by (1) absorption maxima, (2) behavior before and after saponification, (3) partition coefficients between 95% methanol and hexane[3], and (4) co-chromatography with authenic samples. The carotenoid chromoprotein was extracted in 0.1 *M* phosphate buffer (pH 7.2), filtered, and centrifugated at 27,000 *g* for 1 h in the cold to remove non-colloidal particulate matter.

Results. The nature and relative amounts of the carotenoids found are recorded in the Table, together with absorption maxima in hexane and partition coefficients both before and after treatment with alcoholic alkali. Zone 1 did not separate from synthetic β-carotene when the 2 compounds were co-chromatographed. Zone 2 was

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Carotenoids found in *Betaeus harfordi*, listed in order of increasing adsorption on silica gel thin layer

Pigment	% of total	λ_{max} (hexane, nm) A[a]	B[b]	Partition coefficients between hexane and 95% methanol A[a]	B[b]
β-carotene	4.2	470, *442*, ~422	470, *442*, ~422	100:0	100:0
Echinenone	2.2	~476, *450*	–	epiphasic	epiphasic
Astaxanthin ester	37.6	464	466	93:7	25:75
Astaxanthin ester	35.8	464	466	74:26	25:75
Astaxanthin	20.2	464	466	13:87	25:75

[a] Before saponification. [b] After saponification.