THE PHARMACOLOGY OF 6-HYDROXYDOPAMINE 6559

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INTRODUCTION

After Senoh and coworkers had suggested that 6-hydroxydopamine (6-OHDA) might be formed from dopamine (DA) as an autoxidation product or metabolite (1–4), pharmacological studies revealed that systemic administration of 6-OHDA produces an extremely long-lasting depletion of norepinephrine (NE) from peripheral sympathetically innervated organs (5–7). Although the possible mechanism of this long-lasting depletion was the subject of extensive speculations (6–8), the interest in this amine was initially very limited since other long-lasting NE depletors were already known. However, when electron microscopic studies revealed that 6-OHDA produces a selective destruction of peripheral adrenergic nerve terminals (9, 10) this amine became one of the most intensely studied drugs in the last few years.

The fact that in adult animals 6-OHDA destroys the nerve terminals alone and leaves the cell body intact (10–13) providing the possibility for subsequent regeneration (10–14), whereas in newborn animals the whole neuron is destroyed (15, 16), makes this amine a valuable tool for both reversible and permanent sympathectomy. Of even greater interest is the unique possibility of selectively destroying noradrenergic and dopaminergic neurons (preferentially nerve terminals) in the brain by intraventricular or intracerebral injection of 6-OHDA (17–22).

MORPHOLOGY

Peripheral sympathetic nervous systems.—The selective destruction of peripheral adrenergic nerve terminals by 6-OHDA was discovered in the course of fine structural studies designed to localize various phenethylamines by electron microscopy (9–11, 13, 23). Twelve hours to three days after administration of large doses of 6-OHDA to cats and rats the adrenergic nerve terminals of all organs studied are in various stages of degeneration. In many instances the Schwann cells engulf the nerve terminals, which appear to be in the process of lysis. In contrast, all other cells of the same organ seem to be completely intact, particularly the cholinergic nerve terminals. The selectivity of destruction is most impressively demonstrated in organs such as iris and vas deferens, where cholinergic and adrenergic nerve terminals are located very closely, sometimes surrounded by the same Schwann cell (10, 11).

More detailed studies on the time-course of morphological alterations showed that the first degenerative changes are present as early as one hour after intravenous administration of 6-OHDA (24, 25). However, even after a few minutes the vesicles of the adrenergic nerve terminals reveal an accumulation of 6-OHDA and a decrease in their uptake and/or storage capacity for exogenously administered amines, such as 5-hydroxydopamine (24–27).

The alterations in the adrenergic nerve terminals progress gradually and after a few days resemble very closely those found after surgical denervation (28). The completeness of destruction varies from organ to organ and from species to species (11, 14, 24, 25, 29–32). The same is true for the rate of regeneration (10, 11, 30, 33) which ranges from a few days in the rat mesenteric artery (Kuhn & Tranzer, unpublished) to a few months in the cat iris (10).

The cell bodies in sympathetic ganglia of adult cats and rats show no ultrastructural changes after treatment with 6-OHDA, even if very high doses are topically applied either by direct injection into the ganglia or close-arterial perfusion (13, 27). The resistance of the cell body to the damaging effect of 6-OHDA may explain why a virtually complete regeneration of the adrenergic nerve terminals occurs in most instances.

In contrast to its effect on adult animals, 6-OHDA produces a destruction of the whole adrenergic neuron in newborn rats and mice (15, 16, 33–35). This irreversible chemical sympathectomy is generally more complete than immunosympathectomy achieved by administration of nerve growth factor antiserum (16, 36).

Fluorescence microscopic investigations, performed under various experimental conditions in many species, are in agreement with the fine structural findings. They show a long-lasting disappearance of the specific fluorescence in the terminal parts of the nervous network, whereas the fluorescence in the more proximal parts of the axon persists or even increases (12, 24, 30, 33). This increased fluorescence most probably results from the accumulation of NE storage vesicles in the "amputation stump" of the axon as is the case after ligation of postganglionic adrenergic nerves (37–39).

Central nervous system.—In order to reach the central adrenergic neurons 6-OHDA has to be injected into the cerebral ventricles (18) or into the cerebral tissue (17), since it does not cross the blood-brain barrier (5, 7).

Although some conflicting observations have been reported, there is general agreement that large doses of 6-OHDA (e.g. single or multiple intraventricular doses of 300 μg in rats) produce degenerative changes in the nerve terminals of brain areas that are rich in noradrenergic or dopaminergic nerve endings. The time-course and the pattern of the fine structural changes are very similar to those in the periphery and the damaged nerve terminals are also engulfed and digested by surrounding glial cells within a few days (18–20).

In view of the so far insufficient electron microscopic techniques, which do not allow unambiguous distinction of central dopaminergic and noradrenergic nerve terminals from those containing other transmitter substances (20), this

method is unsuitable for establishing the specificity of the destructive effect of 6-OHDA. However, fluorescence microscopic studies revealed that after large doses of 6-OHDA the specific fluorescence of noradrenergic and dopaminergic nerve terminals disappears permanently, i.e. up to two years after administration of 6-OHDA, whereas the fluorescence of the serotoninergic terminals remains unaffected (12, 21, 40). Furthermore, autoradiographic investigations have shown that pretreatment with 6-OHDA abolishes the characteristic accumulation of silver grains in the region of adrenergic nerve terminals after intraventricular injection of ^{3}H-NE, whereas the pattern of ^{3}H-5-hydroxytryptamine accumulation remains unaffected (19).

Although there are numerous similarities between the morphological changes produced by 6-OHDA in the periphery and the central nervous system there are also very marked differences. In the main part of the central nervous system the destroyed nerve endings show no tendency for regeneration over observation periods of up to 2 years (21), although regenerative activities have been reported in the spinal cord both after surgical and chemical lesioning of descending noradrenergic fibers (41).

In adult animals the cell bodies of peripheral adrenergic neurons seem to be very resistant to the destructive effects of 6-OHDA, as far as can be judged from morphological studies (27). In the central nervous system the resistance is not a general one. There are not only marked regional differences between the susceptibility of the cell bodies to 6-OHDA but there are also very marked differences in the time-lag between the injection of 6-OHDA and the occurrence of degenerative changes in the pericaryon. The more delayed changes seem to result from retrograde degeneration (22), whereas the rapidly occurring changes are attributed to the uptake of 6-OHDA at the level of the pericaryon. For instance, degenerative changes in dopaminergic cell bodies of the substantia nigra have been observed as early as 12 hours after local administration of 6-OHDA (21).

In general the noradrenergic cell bodies seem to be more resistant than the dopaminergic ones but there are also very marked regional differences. For example, the dopaminergic cell bodies in the hypothalamus are not affected even after local application of very high doses of 6-OHDA (21, 40), but a single intraventricular dose of 300 μg 6-OHDA produces an almost complete disappearance of the noradrenergic neurons in the locus coeruleus of the rat. The disappearance of these neurons is a typical example of a delayed type of destruction resulting most probably from a progressive retrograde degeneration (22). In view of these recent findings that cell bodies do not degenerate until a few months after administration of 6-OHDA, the results of earlier investigations covering a period of only a few days or weeks may have to be reconsidered.

Biochemical Effects of 6-OHDA

Peripheral effects of 6-OHDA.—After electron microscopic studies had shown that 6-OHDA produces a selective destruction of adrenergic nerve terminals in adult animals, the question arose as to whether the long-lasting NE depletion—

the prominent finding of the initial pharmacological studies (5–7)—is a reliable index for the extent of the destruction of the adrenergic nerve terminals. At least in the rat heart there is a close correlation between the reduction of NE and tyrosine hydroxylase (42), an enzyme that is selectively located in adrenergic neurons (43, 44). This is not only the case for the reduction occurring immediately after administration of 6-OHDA but also for the time-course of subsequent recovery.

The uptake of 6-OHDA into adrenergic nerve endings seems to be an imperative condition for its destructive effect, because drugs interfering with the transport of NE and other phenylethylamines through the neuronal membrane abolish both the accumulation of ^{3}H-6-OHDA in adrenergic neurons and the long-lasting NE depletion together with the characteristic morphological changes (12, 45–47).

Furthermore, a critical dose of 6-OHDA seems to be necessary to produce the characteristic biochemical changes (long-lasting NE depletion, reduction in tyrosine hydroxylase) reflecting the destruction of the adrenergic nerve endings. This critical dose varies very considerably from organ to organ, resulting most probably from the varying blood supply both with respect to the organ mass and the density of innervation (11, 42). The denser the innervation the smaller is the share of the amine delivered by the circulation to the single nerve ending. This might also explain the marked differences in the extent of reversible chemical sympathectomy after repeated administration of large doses of 6-OHDA (11, 42, 49, 50).

The fact that the destruction of the adrenergic nerve terminals depends on a critical dose of 6-OHDA is further supported by experiments with ^{3}H-labelled 6-OHDA (11). Thirty minutes after administration of ^{3}H-6-OHDA the amount of ^{3}H-amines retained in the heart and spleen of the rat corresponds to the dose administered. However, after 2 and even more impressively after 24 hours the amount of H^3-amines retained is inversely related to the dose administered. These findings suggest that 6-OHDA given in low doses is taken up into the adrenergic nerve terminals without producing any detectable damage, acting as a false adrenergic transmitter. However, high doses of 6-OHDA produce damage to functionally important macromolecular elements of the nerve terminals, including the storage vesicles, which then become no longer available to endogenous NE or to the injected ^{3}H-6-OHDA.

As was to be anticipated from experiments with surgical denervation and immunosympathectomy (51), the uptake and retention of ^{3}H-catecholamines after repeated administration of large doses of 6-OHDA is greatly reduced (11, 52–54). However, this reduction refers only to the neuronal uptake whereas the extraneuronal uptake is not impaired (52). In addition to the reduced uptake and retention of ^{3}H-catecholamines in adrenergic neurons there is also a relative increase in the O-methylated and/or deaminated metabolites (11). Furthermore, after administration of ^{3}H-dopamine not only is the neuronal retention of this amine greatly reduced but also its transformation into NE (55), which is in accordance with the observation that not only tyrosine hydroxylase (44) but

also dopamine β-hydroxylase (56) is very markedly decreased in the rat heart after administration of 6-OHDA.

In adult animals, doses of 6-OHDA, which leads to an extensive destruction of adrenergic nerve terminals, produce no detectable ultramorphological changes in the corresponding cell bodies (10, 11, 13). However, recent biochemical investigations have shown that in spite of the absence of morphological changes 6-OHDA is not absolutely devoid of damaging effects on the adrenergic cell bodies in adult animals. Treatment with high doses of 6-OHDA not only does not produce the expected trans-synaptic induction of tyrosine hydroxylase and dopamine β-hydroxylase in the cell body of adrenergic neurons but prevents—at least temporarily—the neuronally mediated induction of these enzymes by administration of reserpine or cold exposure (42, 57). It cannot be decided whether this interference with the trans-synaptic induction of enzymes results from a relatively weak direct damaging effect on the cell body itself or whether the destruction of the nerve terminals is reflected by an impaired or changed function of the pericaryon, manifested by the absence of increased synthesis of specific enzymes by enhanced activity of preganglionic nerves (42, 57).

Both chemical and immunological sympathectomy leave the adrenal medulla unaffected (11, 58), and it is this organ that partially compensates for the destroyed sympathetic neurons and nerve terminals respectively. This compensation manifests itself by an increased synthesis and turnover of adrenal catecholamines and a trans-synaptic induction of tyrosine hydroxylase, and dopamine β-hydroxylase (44, 59, 60), the essential and rate determining enzymes in the synthesis of the adrenergic transmitter (56).

Central effects of 6-*OHDA*.—As in the periphery, the destructive effect is confined to catecholaminergic neurons and is reflected by a selective reduction in the content of NE and DA (18, 61–66), accompanied by a generally corresponding decrease in tyrosine hydroxylase (63–65) and dopamine β-hydroxylase (67). The content of serotonin and γ-aminobutyric acid remains virtually unchanged (62–65). As in the periphery, there are also very marked differences in the degree of catecholamine depletion between different brain regions (18, 63, 64, 68). Doses of 6-OHDA, given intraventricularly, which reduce the catecholamine content of the cortex to $<5\%$, reduce that of the medulla-pons to only 25% (64). These regional differences may result from differences in the concentration of 6-OHDA reaching the single neurons according to their localization in relationship to the site of 6-OHDA administration, i.e. the distance between the neuron and the lumen of the ventricles. However, this cannot fully account for the poor effect on the medulla-pons. In the spinal cord, which is even more remote from the site of injection, the NE depletion is more effective (64, 68), suggesting that the relative resistance of the medulla-pons may reflect the particular richness of cell bodies in this region (69). As stated above, both in the periphery and the central nervous system the cell bodies are generally less susceptible than the nerve terminals (19–21).

In addition to the regional differences between the degree of NE depletion,

there are also very marked differences between the susceptibility of noradrenergic and dopaminergic neurons to 6-OHDA (64, 65). Doses of 6-OHDA, given intraventricularly, which reduce the NE content of the whole brain to 25%, have an immaterial effect on the DA content (64).

The higher susceptibility of noradrenergic nerve terminals provides an opportunity for their relatively selective destruction, leaving the dopaminergic terminals intact. On the other hand, the very efficient protection of noradrenergic neurons by protryptiline and desimipramine can be used for a virtually selective destruction of dopaminergic neurons, since in the presence of these drugs, high doses of 6-OHDA can be administered without producing detectable damage to the noradrenergic neurons (64, 70, 71).

As in the periphery there is generally a good correlation between the long-lasting reduction of the catecholamine content, the reduction of tyrosine hydroxylase, and the reduction of both uptake and retention of intraventricularly injected ^{3}H-NE (64). However, in contrast to the periphery, in the central nervous system under particular experimental conditions, permanent damage of macromolecular constituents, reflected by a permanent decrease in tyrosine hydroxylase activity and catecholamine content have been observed without detectable ultramorphological changes (72, 73).

Functional Consequences of Peripheral and Central Administration of 6-OHDA

The early effects of systemic administration of small and large doses of 6-OHDA have been studied in detail under various experimental conditions by several groups of investigators (74–77). The results can be summarized as follows: Small doses of 6-OHDA produce a short-lasting, indirect sympathomimetic effect resulting from the displacement of NE from the storage sites without causing any other significant functional changes. The administration of large doses of 6-OHDA results in a very long-lasting sympathomimetic effect (76–78), which is calcium-dependent and is accompanied by a gradual deterioration of various specific functions of the neuronal membrane of the adrenergic nerve terminal (76–77). In the cat heart the initial prominent finding is the increased calcium permeability, accompanied by an increased retrograde firing and an increased transmitter release. These first changes are followed by a failure to generate or conduct nerve impulses in response to electrical stimulation of the nerve trunk or chemical stimulation of the nerve terminals by acetylcholine or high potassium concentrations (76, 77). The transport of amines through the neuronal membrane seems to be a rather resistant function, still intact when other membrane functions are already severely impaired.

Repeated administration of high doses of 6-OHDA leads to an extensive destruction of adrenergic nerve terminals in all species studied so far (79). The considerable difference in the extent of destruction from one organ to the other is also reflected by the functional impairment. However, whereas there is a good correlation between the reappearance of nerve terminals and the recovery of the NE content (11, 30, 80), a complete response to sympathetic nerve stimulation

is already reached when the regeneration of the nerve terminals is far from being complete (49, 76). This "premature" recovery of the function is at least partially due to supersensitivity to NE resulting from a decreased reuptake of NE into the nerve terminals, and in some organs also due to an additional nonspecific postjunctional supersensitivity (46, 76, 78, 81, 82). Both pre- and postjunctional supersensitivity are not confined to the periphery but occur also in the central nervous system (83, 84).

If one considers the short- and long-term effects of intraventricular administration of 6-OHDA, it must be remembered that, although 6-OHDA is capable of eliminating the noradrenergic and dopaminergic nerve terminals in large areas of the brain, this elimination is incomplete, and the main part of the cell bodies and nonterminal axons survive (19–21). In view of the incompleteness of destruction and the redundancy of cerebral functional capacity, which may provide compensation for the lost original pathways, the surviving elements may still provide important base line functions.

During the first hours after intraventricular administration of 6-OHDA, rats are sedated and resemble to some extent animals treated with reserpine (66, 85–87). These behavior changes are accompanied by a marked hypothermia (88–90), most probably resulting from the liberation of endogenous NE and involving central α-adrenergic receptors (90). This hypothermia of 3–4°C lasts for a few hours only and is confined to the first injection of 6-OHDA, whereas the hypothermia produced by successive intraventricular injections of NE remains unchanged (90). The initially reduced water and food intake returns to normal after 3–4 days (85).

One of the most prominent changes in behavior after intraventricular injection of 6-OHDA is the increased irritability of rats from being handled (85, 86, 91). This effect is already present after a single dose of 250 μg but becomes more pronounced after further doses of 6-OHDA. There is a significant correlation between the degree of increased irritability and the NE reduction in the brain stem. This increased irritability persists for more than 12 months, the longest period observed so far (86).

The stimulation of motor activity by (+)-amphetamine is not significantly changed by prior administration of two doses of 250 μg 6-OHDA, which reduce the brain catecholamine content to <20% (85). These results, and others, suggest that there is no simple relationship between the level of brain catecholamines and the extent of the response to (+)-amphetamine, although there is good evidence that catecholamines play an important role in the stimulation of motor activity by (+)-amphetamine (92, 93).

Mechanism of Action

The accumulation of 6-OHDA in the adrenergic neuron by the neuronal amine pump seems to be a prerequisite for the destructive effect of this amine (12, 45–47, 64). The fact that the damaging effect of 6-OHDA is mainly confined to the nerve terminals in adult animals and destroys the whole neuron in newborn animals, most probably results from a shift in the transport-efficiency

from the cell body to the periphery during the development from fetal to adult life (79).

While the accumulation in the neuron is an imperative condition for the destructive effect, uptake into the storage sites does not seem to be relevant, as pretreatment with reserpine does not prevent the characteristic ultramorphological and biochemical changes produced by 6-OHDA (24, 27, 94, 95). Similarly the oxidative deamination of 6-OHDA does not appear to be a prerequisite, since administration of inhibitors of monoamine oxidase does not only not prevent the destructive effect but even potentiates it, particularly in the dopaminergic neurons of the central nervous system (71, 95).

With respect to the molecular mechanism of action, recent in vivo and in vitro experiments have provided convincing evidence that (nonenzymatic) oxidation products of 6-OHDA undergo covalent binding with nucleophilic groups of biologically important macromolecules and that the largest density of these covalent bindings and thus denaturation, occurs in the adrenergic neuron where 6-OHDA is selectively accumulated (11, 94, 96, 97). Degeneration occurs as soon as a critical degree of damage is reached. However, it cannot yet be definitely decided whether H_2O_2, formed from the nonenzymatic oxidation of 6-OHDA, is also involved in the damaging effect (11, 98).

Chemical sympathectomy by 6-OHDA is an impressive example of a relatively nonspecific, general reaction at the molecular level, i.e. covalent binding of oxidation products to nucleophilic groups of macromolecules and possibly formation of H_2O_2, leading to a highly specific pharmacological effect by selective accumulation of the drug in a particular cell type by a specific transport mechanism. Very recently similar effects have been reported for several guanidine derivatives, which are accumulated in adrenergic neurons and produce severe damage both in adult and newborn animals (99, 100). Furthermore, intraventricular injection of 5,6-dihydroxytryptamine leads to a destruction of serotoninergic nerve terminals, although the destructive effects of this compound on the serotoninergic neurons does not seem to be as efficient as that of 6-OHDA on the catecholaminergic neurons (101).

6-OHDA AS AN EXPERIMENTAL TOOL

Destruction or functional elimination of an organ to characterize its physiological role is a well established principle that has found wide application in the investigation of the physiology and pharmacology of the sympathetic nervous system (79). The surgical procedure is still the method of choice for denervation of single organs or groups of organs that are innervated from a single, easily accessible ganglion such as the superior cervical. However, for general sympathectomy the surgical procedure is too cumbersome and too time consuming, particularly if a large number of denervated animals is required.

The fact that 6-OHDA destroys the whole neuron if administered to newborn animals but only the nerve terminals when given to adult animals, provides the unique possibility of achieving either a "permanent sympathectomy" by

treating newborn animals, or a "reversible sympathectomy" by treating adult animals (11, 14–16, 42, 79).

Although chemical and immunological sympathectomy are preferable to the surgical procedure for general sympathectomy, these two methods also have their limitations, as there are considerable differences in the effectiveness with which the single organs are denervated. The advantages and drawbacks of the various procedures have been described in detail very recently (79).

Although 6-OHDA has found wide application as a tool for general peripheral sympathectomy (102–109), its intraventricular and intracerebral administration is of even greater interest (40, 66, 79, 84–87, 91, 111–118), since it provides the unique opportunity of destroying a single population of nerve terminals in the functionally and topographically highly complex organization of the central nervous system.

In view of the extraordinary capacity of the brain to compensate functionally for the loss of destroyed elements, it is not so astonishing that the initial optimistic expectations have not been completely fulfilled, above all with respect to the overall role played by the noradrenergic and dopaminergic system in the brain. The situation becomes even more complex when one considers that the damaging effect of 6-OHDA involves not only catecholaminergic nerves but also their receptors (90). Furthermore, noncatecholaminergic neurons may also be damaged by 6-OHDA, if sufficiently high local concentrations are reached (19).

Although the intraventricular administration of 6-OHDA did not completely fulfill initial expectations, it has provided useful biochemical and morphological information. The fact that repeated administration of 6-OHDA produces a permanent increased irritability (86, 91), which is accompanied by characteristic changes in the turnover rate of the transmitter in the residual neurons, both of which can be normalized by administration of benzodiazepines, provides a valuable tool for the study of psychopharmacological drugs (86). However, the influence of intraventricular injection of rats with 6-OHDA on the subsequent administration of neuroleptic and antidepressive drugs is rather complex and ambiguous (110).

Of particular interest is the selective elimination of specific parts of the central noradrenergic or dopaminergic system to elucidate their physiological importance in the regulation of behavior, sleep, endocrine, and autonomous functions (40, 111, 117).

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