Biochemical Pharmacology, Vol. 24, pp. 1–8. Pergamon Press, 1975. Printed in Great Britain.

# COMMENTARY

## MECHANISMS OF ACTION OF 6-HYDROXYDOPAMINE*

CHARLOTTE SACHS and GÖSTA JONSSON
Department of Histology, Karolinska Institutet, 104 01 Stockholm, Sweden

With the discovery of the unique properties of 6-hydroxydopamine (6-OH-DA) a new principle for experimentally induced degeneration of specific neuron systems was introduced into neurobiology, i.e. "chemical denervation" [1]. This principle is based on 6-OH-DA being a generally cytotoxic substance that is taken up and concentrated by a specific transport mechanism of catecholamine (CA) neurons, the "membrane pump". Since this process leads to an efficient intraneuronal accumulation of 6-OH-DA its cytotoxic effects can be rather selectively restricted to CA neurons (see symposium volume on 6-OH-DA [2]). The purpose of this Commentary is to review and discuss present state knowledge regarding the mode of action of 6-OH-DA, factors modifying its toxicity and specificity, since these aspects are of great significance in the use of 6-OH-DA as a denervation tool.

### MODE OF ACTION

There are two main theories on the degenerative action of 6-OH-DA at the molecular level, both associated with the susceptibility of 6-OH-DA to non-enzymatic oxidation (see Fig. 1). 1. Oxidation products of 6-OH-DA (quinones) may undergo covalent binding with nucleophilic groups of macromolecules, such as SH, $NH_2$ and phenolic OH. Such bindings would cause denaturation of molecules of vital importance for the integrity of the neuron leading to an irreversible damage and subsequent degeneration [3, 4]. 2. Hydrogen peroxide ($H_2O_2$) is formed during the oxidation of 6-OH-DA and it is assumed that $H_2O_2$ may be responsible for the nerve degeneration [5, 6]. It has also been observed that $H_2O_2$ formation is markedly enhanced in the presence of ascorbic acid. The degenerative action may in this case be brought about by oxidation of important sulfhydryl groups of enzymes and membranes or peroxidation of membrane lipids. Recently the very interesting observation has been made that superoxide and hydroxy radicals are produced during the autooxidation process of 6-OH-DA [7, 8]. These radicals may not only be cytotoxic *per se*, but there are also indications that the superoxide radical plays an important role in controlling the over-all rate of oxidation of 6-OH-DA. It has thus been found that the superoxide radical promotes the oxidation of 6-OH-DA resulting in an increase in the formation of radicals, $H_2O_2$ and quinones (see Fig. 1). Of great interest are the observations obtained from *in vitro* experiments that the CA, noradrenaline (NA) and dopamine (DA) may trap these radicals and consequently retard the autooxidation of 6-OH-DA [8].

It has been much debated whether 6-OH-DA itself or any of its metabolite(s) or oxidation product(s) is the effective degenerative agent. It seems likely that 6-OH-DA must be intact initially in order to be taken up and accumulated intraneuronally. Consistent with this view it has also been found that the neurotoxic effects of 6-OH-DA are abolished after vigorous oxidation of 6-OH-DA in NaOH [9]. However, the administration of the pure *para*- and *ortho*-quinones of 6-OH-DA has been reported to cause degeneration of adrenergic nerves similar to 6-OH-DA [10]. These quinones show an equal neurotoxic potency, but are clearly less effective than 6-OH-DA itself. Similar to 6-OH-DA the neurotoxic effects of the quinones can be blocked after inhibition of the "membrane pump" by desipramine and potentiated by ascorbic acid and monoamine oxidase (MAO) inhibition. It has also

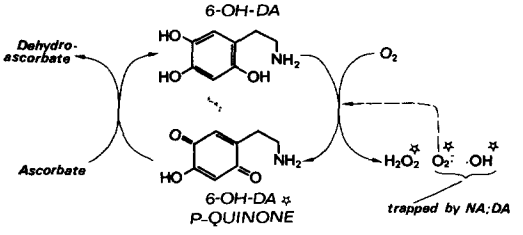


Fig. 1. Hypothetical route of reaction for the autooxidation of 6-OH-DA. A *para*-quinone of 6-OH-DA, $H_2O_2$ and superoxide ($O_2^-\cdot$) and hydroxy ($\cdot OH$) radicals are formed by the oxidation of 6-OH-DA. The superoxide radical catalyses the oxidation of 6-OH-DA, with a consequent increase in the production of quinones, $H_2O_2$ and radicals. 6-OH-DA may be regenerated from the *para*-quinone by the oxidation of ascorbate. Radicals can be trapped in the presence of NA or DA. The possible cytotoxic products are marked with an asterisk. No distinction between all possible oxidized forms of 6-OH-DA has been attempted in this schematic representation (after Heikkila and Cohen [6, 10]).

* Supported by The Swedish Medical Research Council, 04X-3881 and 04X-2295.

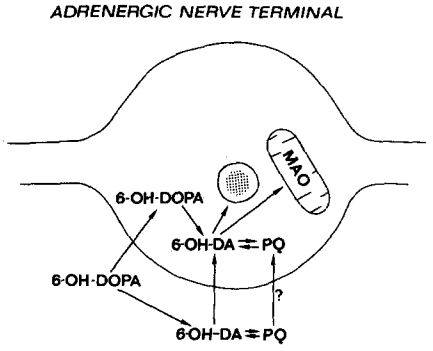


Fig. 2. Schematic representation of possible events at the nerve terminal after administration of 6-OH-DOPA, 6-OH-DA or *para*-quinone (PQ,). 6-OH-DOPA may, as an amino acid, be taken up directly by the nerve terminal and then intraneuronally decarboxylated to 6-OH-DA. Alternatively, 6-OH-DOPA may be decarboxylated outside of the nerve to 6-OH-DA, which is taken up and concentrated by the "membrane pump", identical to the situation after administration of 6-OH-DA itself. 6-OH-DA may be oxidized to its PQ and again recycled to 6-OH-DA, which is taken up by the membrane pump (see Fig. 1). Inside the nerve terminal 6-OH-DA can be taken up by the amine storage granules and/or degraded by MAO. After administration of PQ, this compound may either be taken up directly or after extraneuronal transformation to 6-OH-DA (for details, see text).

been found that the quinones possess a lower affinity to the uptake sites of the adrenergic nerves compared with 6-OH-DA (unpublished results). The marked potentiating effect of ascorbic acid indicates that the quinones have to be transformed to 6-OH-DA before eliciting the neurotoxic action. However, it is not known whether the transformation occurs intra- or extraneuronally and thus not clear if the quinones themselves are taken up by the adrenergic nerves to cause degeneration or if first they are transformed to 6-OH-DA, which then is taken up and accumulated by the neuron (Fig. 2). Consequently it is also not possible to conclude anything from these results concerning the quinones as being the causative agents for inducing the neuronal damage. Apart from quinones there are also other products that can be formed from 6-OH-DA, e.g. 5,6-dihydroxyindole [11]. This latter compound has also been suggested to be an effectual agent in the degeneration process.

The autooxidation of 6-OH-DA is thus a complex reaction with a simultaneous formation of several possible cytotoxic products, which even may interact between themselves and the neurotransmitter, making it very difficult experimentally to establish which of them is the most important for initiating the degeneration.

Other contributary mechanisms for producing the nerve degeneration have also been put forward. There is such an extremely rapid oxygen consumption during the autooxidation of 6-OH-DA that a certain degree of hypoxia may occur within the neuron [6]. Another deleterious action of 6-OH-DA may be associated with its property of being a very potent uncoupler of oxidative phosphorylation [12].

## EFFECTS OF 6-OH-DA ON THE ADRENERGIC NEURON

As already mentioned, the specificity of the action of 6-OH-DA is associated with its uptake and accumulation by a transport mechanism specific for CA neurons, since inhibition of this uptake mechanism completely prevents the neurotoxic effects [13, 9]. The importance of the specific accumulation for the degenerative action is amply illustrated by experiments with 6-OH-NA. This compound undergoes like 6-OH-DA a rapid autooxidation with production of $H_2O_2$ and radicals and should therefore be expected to be a neurotoxic compound for CA neurons. However, 6-OH-NA has a very low affinity for the transport sites of the "membrane pump" and is therefore not concentrated in adrenergic nerves. Consequently no selective neuronal degeneration has been observed, but only some unspecific tissue damage [14, 15].

A threshold concentration of 6-OH-DA must be reached within the neuron in order to elicit the degeneration. This critical 6-OH-DA concentration has been calculated to be in order of 5,000 μg/g, as evaluated from *in vitro* experiments with $^3$H-6-OH-DA [16]. This figure is in the same order of magnitude as the endogenous NA concentration in the adrenergic nerve terminal, but it is so far not known whether it is directly applicable to the *in vivo* situation after 6-OH-DA administration.

From *in vivo* experiments it has been found that 6-OH-DA acts on the adrenergic nerves in an "all or none" fashion with a complete destruction of NA transmitter mechanisms, such as NA uptake–storage, in a proportion of nerves depending on the dose used [17, 18]. The remaining nerves are left with mainly intact transmitter mechanism. Displacement of NA seems to play a minor role in the NA depleting action and only at a very low dose range after which 6-OH-DA can serve as a false transmitter [19, 18]. In most studies very little evidence has been obtained for 6-OH-DA causing transient damage not associated with degeneration, although it has been reported that 6-OH-DA in the low dose range may produce transient functional damage [19]. It has been claimed that this effect can be counteracted by catalase [20].

The axonal membrane functions are impaired at a very early stage in the degeneration process. There is thus within one hour a loss of the ability of the adrenergic nerve terminals to conduct and to generate action potentials, concomitant with a release and disappearance of NA [21]. A permanent depolarization of the axonal membrane by 6-OH-DA, associated with increased $Ca^{2+}$-permeability has been assumed to be the cause. Consonantly, a damage of the NA uptake mechanism at the axonal membrane is an early sign in the degeneration process [9, 17, 18]. The end results

are similar to those seen after surgical denervation, with development of both pre- and postsynaptic supersensitivity. 6-OH-DA has also been found to block α-adrenergic receptors, although the information on this point is relatively incomplete [21, 23]. There is a parallel disappearance of the endogenous NA content [19, 9] and of the activity of enzymes engaged in the NA biosynthesis, such as tyrosine hydroxylase and DOPA decarboxylase [24].

The apparent neurotoxic potency of 6-OH-DA *in vivo* varies markedly in various parts of the sympathetic adrenergic neuron. The nerve terminals have been found to be most sensitive, the axons less and the cell-bodies least sensitive to 6-OH-DA, which are generally not affected at all [13, 9]. The cell-bodies are ultrastructurally unchanged, even when a high concentration is injected directly into the ganglion [25], although a drop in the synthesis of DA-β-hydroxylase in the ganglia has been noted after systemic administration of a large dose of 6-OH-DA [26]. The latter observation has been interpreted as due to a shift in protein synthesis from "functional" proteins, e.g. enzymes for the NA biosynthesis, to structural proteins necessary for neuronal regeneration. Incubation of isolated ganglia *in vitro* in a high 6-OH-DA concentration (0·1 mM, 60 min) has been found to produce a marked NA depletion, but such a high concentration for a long time may be difficult to reach *in vivo* without causing general toxic effects [9]. It is clear, however, that cell-bodies can undergo degeneration when 6-OH-DA is injected to newborn rats, leading to a permanent, but not complete sympathectomy [27]. It is not known whether the susceptibility in the early stage of development is due to the neuron being more sensitive *per se* or to other factors, e.g. less developed connective tissue barriers, making more 6-OH-DA accessible for the axon membrane (see below).

Degenerative signs, like axonal swelling and shrinkage of the dense-core vesicles, can be seen by electron-microscopy in the nerve terminals already within 1 hr after a 6-OH-DA injection. Between 2 and 4 hrs electron-dense axonal debris within Schwann cells begins to appear, indicating that the digestive process has started [28, 29]. The degenerative signs at the ultrastructural level are very similar to those seen after surgical axotomy, i.e. dense type of degeneration, but since 6-OH-DA has a direct action on the terminals, the degeneration appears faster after 6-OH-DA [30, 29]. The adrenergic nerve terminals have completely vanished after 48–72 hr. Electron dense bodies in adrenergic terminals have been observed also after *in vitro* incubation with 6-OH-DA [29].

By fluorescence histochemistry according to Falck-Hillarp there is a dose-dependent reduction of the number of NA containing terminals. An indication of nerve degeneration at the light microscopical level is the appearance of NA accumulations in the main axons [13, 31, 32]. This phenomenon, which is also seen after surgical lesions is related to a piling up of transmitter, transported by the axoplasmic flow, proximal to the site of the lesion [33]. At times the fluorescent accumulations can be rather longish and more pronounced than after a surgical axotomy, indicating a more diffuse damage of structures associated with the axoplasmic flow, e.g. the neurotubules [34].

The exact reasons for the apparent differences in sensitivity *in vivo* to 6-OH-DA in various parts of the neuron are not known, but there are several factors that could contribute. Variations in blood-circulation, diffusion conditions, e.g. different connective tissue barriers and/or differences in affinity and transport of 6-OH-DA by the "membrane pump" [35] could be responsible for the observed differences in sensitivity. Another significant factor may be differences in the surface/volume relationship, which is rather marked for cell-bodies and nerve terminals. Considering this relationship for a ganglion cell (volume $10–20 \times 10^3$ $\mu m^3$, diameter 50 $\mu m$ [36]) and a varicosity (0·52 $\mu m^3$, diameter 1 $\mu m$), it would thus be an 8–15 times more favourable situation for the varicosity of reaching a critical intraneuronal 6-OH-DA concentration, assuming that the blood-circulation, diffusion conditions and 6-OH-DA affinity are the same for both structures.

Since the cell-bodies mostly are left apparently unaffected by 6-OH-DA, a regeneration of nerve fibers may occur. The regeneration is more rapid after a lower dose of 6-OH-DA compared with a higher, even when the extent of degeneration of the terminal nerve plexus initially is the same and complete [37]. This finding may be explained by a variation in axonal damage, i.e. with the lower dose mainly the terminals are destroyed while with a higher dose also the axons are more or less damaged. This would then lead to divergences in the rate of regeneration, although it cannot be excluded that the higher dose also affects the perikaryon.

6-OH-DA can thus be used for studies on regeneration and growth of neurons and several such studies have been performed in the peripheral and central nervous system [9, 38, 39].

## FACTORS INFLUENCING THE DEGENERATION BY 6-OH-DA

Besides "extraneuronal factors" such as blood-circulation, diffusion conditions and surface/volume relationships, there are also certain "intraneuronal factors" which can modulate the degenerative action of 6-OH-DA. It is obvious that if the "membrane pump" function various under different conditions, this would lead to a varying degenerative effect. However, except for the use of experimental conditions that inhibit this uptake mechanism (e.g. desipramine, 0°) with an accompanying prevention of the degeneration, very little is known. It is possible that the reduced effects of 6-OH-DA seen after pentobarbital anaesthesia represents such a modification of the transport of 6-OH-DA into the neuron [40].

Intraneuronally 6-OH-DA can be taken up and stored by the amine storage granules, probably via the

reserpine-sensitive ATP-$Mg^{2+}$ dependent uptake mechanism [9, 41]. Blockade of the granular uptake–storage mechanism by reserpine has no or very small effects on the 6-OH-DA induced degeneration [9, 13]. Therefore it may be concluded that an intact granular uptake–storage mechanism is not a prerequisite for the degenerative action. This would also mean that the critical 6-OH-DA concentration that has to be reached intraneuronally to induce degeneration is more directly related to that obtained in the extragranular space. In agreement with this view, it has been observed that amphetamine, in a dose considered to cause mainly a release of extragranular NA, reduces the neurotoxic effects of 6-OH-DA [42]. This is best explained by amphetamine causing a release of 6-OH-DA present in the axoplasm leading to a diminished degeneration. Although these results point to the extragranular localization of 6-OH-DA as the most important site of action, the role of the amine storage granules is still not quite clear. It is possible that the storage granules, under certain conditions either could have a protective role by removing 6-OH-DA from the axoplasm or contribute to maintain the high 6-OH-DA concentration within the nerve necessary to produce degeneration [43].

Recent findings by Heikkila and Cohen [7, 8] indicate that the transmitter content within the CA neurons may be an important factor in modifying the neurotoxic action of 6-OH-DA (see above). It has thus been found that during the autooxidation of 6-OH-DA radicals are formed, which may be cytotoxic and also control the overall oxidation rate of 6-OH-DA. Since these radicals can be trapped by CA, the transmitter itself may counteract the effects of 6-OH-DA. Accordingly experiments *in vivo* have shown that adrenergic nerve terminals with an increased NA content were partially protected from neurotoxic effects of 6-OH-DA, whereas a reduced NA content led to a potentiation of the degeneration (unpublished results). The intraneuronal NA concentration seems to modify the degenerative effect, which may explain some of the differences in sensitivity to 6-OH-DA seen in various CA neuron systems (see below).

If the autooxidation of 6-OH-DA is causally related to the neurotoxic action of 6-OH-DA, which the information so far available strongly supports, the local concentrations of enzymes such as catalase, glutathione reductase and superoxide dismuthase may be significant factors in regulating the neurotoxicity of 6-OH-DA. However, very little is known on this point.

Inside the neuron 6-OH-DA can be attacked by monoamine oxidase (MAO) and inhibition of this enzyme leads to an increased degenerative effect [42]. This potentiation is especially pronounced when using low doses of 6-OH-DA, which is consistent with the view that a threshhold concentration of 6-OH-DA must be reached for eliciting the degeneration. However, when using 6-OH-DA as a denervation tool, MAO inhibition is of very little importance since supramaximal doses are ordinarily used. These data indicate in addition, that the deaminated metabolites of 6-OH-DA are of little, if any, significance in the neurotoxic action of 6-OH-DA.

When working with 6-OH-DOPA, the immediate precursor of 6-OH-DA, MAO inhibition is of great significance for the neurotoxic potency [44]. The degenerative effects of 6-OH-DOPA are mediated via 6-OH-DA, formed by decarboxylation of 6-OH-DA, either extra- or intraneuronally (see Fig. 2). Systemic injections of 6-OH-DOPA can be used for CNS studies, since it passes the blood-brain barrier [44, 45]. A limiting factor when using 6-OH-DOPA, being an amino acid, is that relatively high doses have to be used in order to obtain a substantial degeneration of NA nerves, which is not possible without causing general toxicity and death. However, inhibition of MAO leads to a considerable potentiation of the degenerative effect which may be related to a relatively slow decarboxylation process of 6-OH-DA (see Fig. 2).

The information on the possible importance of the nerve impulse flow for the degenerative action is very limited. However, decentralization of the submaxillary gland and iris has been reported to have no effect on the degeneration process [46].

## HETEROGENEITY OF THE ACTION OF 6-OH-DA ON CA NEURONS

From the previous section it is clear that the neurotoxicity of 6-OH-DA can be regulated at many different levels, therefore it may not be surprising that there is a considerable heterogeneity in the response to 6-OH-DA in different CA neuron systems. It was soon realized that the apparent neurotoxic potency of 6-OH-DA varied markedly in different peripheral organs [9, 13, 19]. The nerve terminals in the vas deferens are thus rather resistant to 6-OH-DA compared with those in the iris. Apart from possible differences in blood-circulation and diffusion conditions, this variation in sensitivity may be related to the fact that the endogenous NA content in nerve terminals of the vas deferens is about 30% higher than in terminals of the iris [47]. If the intraneuronal NA concentration is a modifying factor, which is very likely (see above), then neurons with a higher NA concentration would be more resistant to 6-OH-DA. This effect might also partly explain the observed difference in sensitivity to 6-OH-DA between NA nerve terminals in the cerebral cortex and the hypothalamus [44, 48, 49, 50]. Independent of the route of administration, the cortical nerve terminals seem to be more sensitive. The two brain areas are innervated by two different ascending NA pathways: The cerebral cortex by a dorsal NA bundle, originating from cell-bodies in the *locus coeruleus* in pons, and the hypothalamus by a ventral NA bundle, originating from cell groups in the lower brainstem [51, 52]. The nerve terminals of these systems differ in their NA fluorescence morphology. The nerve terminals of the cerebral cortex are small with low

fluorescence intensity (= low NA concentration), while in the hypothalamus there are at least two populations; the majority is of a coarse type with strong fluorescence intensity (= high NA concentration) and the rest is indistinguishable from those in the cerebral cortex. The sensitivity differences could thus be related to the observed differences in intraneuronal amine concentration, as well as in surface/volume relationships between the terminals of the cortex and of the hypothalamus [53].

There are also differences in sensitivity to 6-OH-DA among various groups of CA perikarya, which in general are very resistant. However, after local injection of 6-OH-DA into the *substantia nigra*, the DA perikarya with the nerve terminals belonging to them can undergo permanent degeneration [54, 55]. As regards the NA perikarya, these have in the majority of reports been found to be unaffected by any type of 6-OH-DA administration and dose. However, after a very high dose of 6-OH-DA intraventricularly, the NA perikarya of the *locus coeruleus* have been reported to completely disappear [56], but it is not quite clear whether this is related to a specific neurotoxic action 6-OD-DA or not, since the ventricles were found to be considerably dilated. In general it seems as if the NA perikarya are more resistant than DA perikarya, although there is at present no accurate explanation for this difference. Among the known DA neuron systems it has been noted after local 6-OH-DA injection that the nigro-neostriatal system is most sensitive, the mesolimbic somewhat less sensitive, while the tuberoinfundibular DA neurons are almost unaffected by the degenerative effects of 6-OH-DA [57]. It is not known if this is related to true differences in susceptibility of the DA neurons themselves, and/or to differences in their neuroanatomical localization with possible variations in the diffusion conditions for 6-OH-DA.

After intraventricular injection of 6-OH-DA, the DA content of the brain is less reduced than the NA [24]. In agreement with this finding introduction of 6-OH-DA into the brain via systemic or intraventricular injection of 6-OH-DOPA causes no effects on DA neurons, unless extremely high doses are used [31, 44, 45]. It has also been noticed that systemic injections of 6-OH-DA in high doses to newborn rats, before the BBB is developed will leave the DA neurons largely intact, while the NA nerve terminals, especially in the forebrain, are to a great extent degenerated [48, 58]. There is no clear-cut explanation for this difference between NA and DA neurons, but ultimately it must be due to variations in obtaining the necessary critical intraneuronal concentration of 6-OH-DA. Differences in the affinity of 6-OH-DA to the transport sites has been suggested to be the cause, since 6-OH-DA *in vitro* has been noted to have a higher affinity to the "membrane pump" of NA than of DA nerve terminals [59]. Other contributing factors may be topographical variations in the neuroanatomy and/or differences in the intraneuronal amine concentrations or subcellular distribution of the transmitter in the two neuron types. This latter possibility may be of significance in view of the evidence pointing to the neurotoxic action of 6-OH-DA mainly taking place in the axoplasm outside of the granules and relatively more amine is present extragranularly in the DA neurons compared with the NA neurons [60]. It is therefore possible that there is a more marked interaction with the autooxidation of 6-OH-DA in the DA neurons than in the NA neurons that would at least partly explain the differences in response to 6-OH-DA between the two neuron types.

For the sake of completeness it should also be mentioned that there are considerable species differences in the neurotoxic potency of 6-OH-DA, but very little is known about the exact reason(s) for this. The sympathetic adrenergic nerves of mice are thus more sensitive than those of rat compared on a mg/kg dose basis [61].

## SPECIFICITY OF 6-OH-DA

In the peripheral nervous system the selective degenerative action of 6-OH-DA on sympathetic adrenergic neurons is undisputed. 6-OH-DA thus leaves cholinergic neurons [1, 29] and certain monoamine containing cells, such as mast cells, SIF cells in ganglia, enterochromaffin cells and adrenal medullary cells unaffected, certainly related to these structures lacking a concentration mechanism for 6-OH-DA [9, 13, 62], but neuroblastoma cells have been reported to be selectively damaged due to their capability of accumulating 6-OH-DA [63]. However, upon systemic administration of large doses of 6-OH-DA certain general toxic effects have been noticed such as hemolysis and damage of tubule cells in the kidney [64]. In this context it may be of interest to mention that the blood-brain barrier in adult rats does not completely protect central NA neurons from the neurotoxic action of 6-OH-DA after systemic administration [65]. Following intravenous injection of 6-OH-DA there is thus a significant long-lasting reduction of NA in the cerebral cortex and the spinal cord, while the DA neurons appear unaffected.

In the central nervous system the degree of selectivity of 6-OH-DA seems to be dependent on its mode of administration. The 5-HT neurons are generally completely unaffected by any type of 6-OH-DA administration [24, 66], except in the cat where a small degenerative effect has been observed [67]. Although this latter observation represents a species difference, it may not be so surprising in view of the fact that 5-HT neurons have an axonal "membrane pump" that is not absolutely specific for 5-HT, but CA also have a certain affinity for the 5-HT transport mechanism [68]. However, in the rat there is a low affinity of 6-OH-DA to the uptake sites of central 5-HT neurons [69] consistent with the finding that 6-OH-DA has very small or no degenerative effects on 5-HT neurons in this species. On the other hand, if the NA neurons are protected by desipramine, 6-OH-DA

administered in the neonate stage may produce a selective 40 per cent reduction of 5-HT, when analysed in the adult stage [70].

As regards the intraventricular route of 6-OH-DA administration, there is an almost exclusive effect on CA neurons, while there are no effects on brain concentrations of a number of putative transmitters such as acetylcholine, γ-aminobutyric acid, glycine and several other amino acids, even when very high doses (2 × 400 μg) are used [40]. The effects of 6-OH-DA on CA neurons can thus be regarded as quite specific, although a certain unspecific damage of the ependymal cells can be seen after such intraventricular doses of 6-OH-DA, which may lead to hydrocephalus [56, 71]. The ependymal cells will initially be exposed to very high 6-OH-DA concentrations, calculated to be in the order of 1–10 mM when the commonly used dose of 200 μg is injected.

6-OH-DA can also be introduced into the brain by systemic injection of 6-OH-DA to newborn animals [48, 58] or via systemic 6-OH-DOPA [44, 54] administration. Both these procedures cause a selective neurotoxic action on NA neurons. After 6-OH-DOPA cholinergic neurons have thus been observed to be unaffected, but unspecific lesions have been seen in the area postrema, in all probability due to the very high local concentrations of 6-OH-DA and/or 6-OH-DOPA that can be reached in this highly vascularized region [31].

Although the information present available points to a rather selective action of 6-OH-DA on CA neurons after intraventricular and systemic administrations, it is obvious for several reasons that these modes of administration possess serious limitations when used for the functional analysis of the various central CA neuron systems [73]. For this purpose local intracerebral injections of 6-OH-DA are advantageous, provided specific lesions can be obtained. However, the results reported using this administration procedure are very inconsistent, which has led to rather contradictory and confusing views as regards the specificity of 6-OH-DA. It is clear that the risks for obtaining unspecific tissue damage are far greater with the local injection technique compared with the other routes of administration. Nevertheless, there is good experimental evidence that a selective degenerative effect can be obtained when 6-OH-DA is injected into neurochemically rather homogenous brain areas [55, 74]. Certain CA neuron systems may thus be selectively destroyed with limited unspecific tissue damage, e.g. the nigroneostriatal DA system [55, 74, 75] and the NA neuron system in the cerebral cortex [76]. Hökfelt and Ungerstedt reported in a recent study only little unspecific damage in most animals after 6-OH-DA injection into the *substantia nigra*, although occasionally fairly large lesions were seen, about 1 mm in diameter including all cellular elements surrounding the site of injection. However, other authors have regularly obtained large, 1–2 mm necrotic areas with unspecific cell damage [71, 77–79]. These latter observations have led Poirer *et al.* and Butcher *et al.* to conclude that in the CNS lesions produced by injection of 6-OH-DA are not more selective than electrolytic or any mechanical or chemical lesion.

Apart from species differences, the varying results reported must in some way be related to differences in the injection techniques used. Even when the dose of 6-OH-DA, the solvent, the solvent volume and the speed of injection are identical, there are probably other factors which can be of importance, such as the degree of purity of the 6-OH-DA, the size and the direction of the opening of the cannula. Various brain regions may also differ in their sensitivity to the unspecific damage of 6-OH-DA, e.g. the mesencephalic reticular formation is consistently more unspecifically damaged than the mesencephalic grey [75]. The concentration of ascorbic acid used as an antioxidant in the solvent may also be of significance, since $H_2O_2$ can be spontaneously formed from ascorbic acid, which in addition potentiates the formation of $H_2O_2$ from 6-OH-DA [5]. Pretreatment of animals with a large dose of ascorbic acid has also been found to augment the degenerative effect of 6-OH-DA on sympathetic adrenergic nerves [80]. Furthermore, in tissue culture even 0·2 mg/ml of ascorbic acid alone causes damage to growth cones of nerve fibers to the same extent as 6-OH-DA [81]. It is therefore likely that the use of high concentrations of ascorbic acid in the solvent, either by itself or together with 6-OH-DA, will substantially enhance the unspecific tissue damage.

Obviously, 6-OH-DA may cause damage to any tissue if a high enough concentration is used, with an effect which presumably would not be very different from a direct application of $H_2O_2$. The uniqueness of the action of 6-OH-DA, by its selective concentration in CA nerves, should be used to maximum advantage and the experiments designed with this in mind. Unspecific damage should, whenever possible, be looked out for with e.g. histological techniques. Even when all precautions are taken in order to make a selective lesion by 6-OH-DA the neuroanatomical situation and/or the diffusion conditions may make it impossible. However, even if the selectivity of the action of 6-OH-DA has been seriously questioned, there is convincing evidence showing that 6-OH-DA properly used can produce a selective degeneration of certain CA neuron systems.

## REFERENCES

1. J. P. Tranzer and H. Thoenen, *Naunyn-Schmiedebergs Arch. Pharmak. exp. Path.* **257**, 343 (1967).
2. T. Malmfors and H. Thoenen (Eds), *6-Hydroxydopamine and Catecholamine Neurones*. North-Holland, Amsterdam (1971).
3. H. Thoenen, J. P. Tranzer and G. Häusler, *Bayer-Symposium II*, p. 130. Springer, Berlin (1970).
4. A. Saner and H. Thoenen, *Molec. Pharmac.* **7**, 147 (1971).

5. R. Heikkila and G. Cohen, *Science, N.Y.* **172,** 1257 (1971).
6. R. Heikkila and G. Cohen, *Molec. Pharmac.* **8,** 241 (1972).
7. R. Heikkila and G. Cohen, *Science, N.Y.* **183,** 456 (1973).
8. G. Cohen and R. Heikkila, *J. biol. Chem.* **249,** 2447 (1974).
9. G. Jonsson and Ch. Sachs, *Eur. J. Pharmac.* **9,** 141 (1970).
10. R. Heikkila, C. Mytilineou, L. Côté and G. Cohen, *J. Neurochem.* **20,** 1345 (1973).
11. C. L. Blank, P. T. Kissinger and R. N. Adams, *Eur. J. Pharmac.* **19,** 391 (1972).
12. K. Wagner and U. Trendelenburg, *Naunyn-Schmiedebergs Arch. Pharmak.* **269,** 112 (1971).
13. T. Malmfors and Ch. Sachs, *Eur. J. Pharmac.* **3,** 89 (1968).
14. Ch. Sachs, *Eur. J. Pharmac.* **20,** 149 (1972).
15. J. Lundström, H. Ong, J. Daly and C. R. Creveling, *Molec. Pharmac.* **9,** 505 (1973).
16. G. Jonsson and Ch. Sachs, *Eur. J. Pharmac.* **16,** 55 (1971).
17. J. L. Bell, L. L. Iversen and N. J. Uretsky, *Br. J. Pharmac.* **40,** 790 (1970).
18. G. Jonsson and Ch. Sachs, *J. Pharmac. exp. Ther.* **180,** 625 (1972).
19. H. Thoenen and J. P. Tranzer, *Naunyn-Schmiedebergs Arch. Pharmak. exp. Path.* **261,** 271 (1968).
20. J. Forn, J. Martin, A. Llorente, L. Casasnovas and F. G. Valdecasas, *Arch. int. Pharmacodyn.* **206,** 84 (1973).
21. G. Haeusler, *J. Pharmac. exp. Ther.* **178,** 49 (1971).
22. G. Haeusler, W. Haeffely and H. Thoenen, *J. Pharmac. exp. Ther.* **170,** 50 (1969).
23. K. Nakamura and H. Thoenen, *Eur. J. Pharmac.* **16,** 46 (1971).
24. N. J. Uretsky and L. L. Iversen, *Nature, Lond.* **221,** 557 (1969).
25. J. P. Tranzer, in *6-Hydroxydopamine and Catecholamine Neurons* (Eds. T. Malmfors and H. Thoenen), p. 351. North-Holland, Amsterdam (1971).
26. S. Brimijoin, *J. Pharmac. exp. Ther.* **183,** 298 (1972).
27. P. U. Angeletti and R. Levi-Montalcini, *Proc. natn. Acad. Sci. U.S.A.* **65,** 114 (1970).
28. J. B. Furness, G. R. Campbell, S. M. Gillard, T. Malmfors, J. L. S. Cobb and G. Burnstock, *J. Pharmac. exp. Ther.* **174,** 111 (1970).
29. T. Hökfelt, G. Jonsson and Ch. Sachs, *Z. Zellforsch.* **131,** 529 (1972).
30. C. D. Roth and K. C. Richardson, *Amer. J. Anat.* **124,** 341 (1969).
31. Ch. Sachs, G. Jonsson and K. Fuxe, *Brain Res.* **63,** 249 (1973).
32. Ch. Sachs, Ch. Pycock and G. Jonsson, *Med. Biol.* **52,** 55 (1974).
33. A. Dahlström, M.D. Thesis, Stockholm (1966).
34. T. B. Cheah, L. B. Geffen, B. Jarrott and A. Ostberg, *Br. J. Pharmac.* **42,** 543 (1971).
35. I. J. Kopin and S. D. Silberstein, *Pharmac. Rev.* **24,** 245 (1972).
36. K.-A. Norberg and B. Hamberger, *Acta physiol. scand.* **63,** Suppl. 238, 1 (1964).
37. G. Jonsson and Ch. Sachs, *J. Neurochem.* **19,** 2577 (1972).
38. L.-G. Nygren, L. Olson and Å. Seiger, *Histochemie* **28,** 1 (1971).
39. J. De Champlain, *Can. J. Physiol. Pharmac.* **49,** 345 (1971).
40. B. R. Jacks, J. De Champlain and J.-P. Cordeau, *Eur. J. Pharmac.* **18,** 353 (1972).
41. T. Bennett, G. Burnstock, J. L. S. Cobb and T. Malmfors, *Br. J. Pharmac.* **38,** 802 (1970).
42. G. Jonsson, T. Malmfors and Ch. Sachs, *Res. Commun. Chem. Path. Pharmac.* **3,** 543 (1972).
43. G. Jonsson, and Ch. Sachs, *Res. Commun. Chem. Path. Pharmac.* **5,** 287 (1973).
44. Ch. Sachs and G. Jonsson, *J. Neurochem.* **19,** 1561 (1972).
45. D. Jacobowitz and R. Kostrzewa, *Life Sci.* **10,** 1329 (1971).
46. H. Goldman and D. Jacobowitz, *J. Pharmac. exp. Ther.* **176,** 119 (1971).
47. A. Dahlström, J. Häggendal and T. Hökfelt, *Acta physiol. scand.* **67,** 289 (1966).
48. Ch. Sachs and G. Jonsson, *Res. Commun. Chem. Path. Pharmac.* **4,** 203 (1972).
49. G. Jonsson and Ch. Sachs, *Biochem. Pharmac.* **22,** 1709 (1973).
50. P. Lidbrink, *Brain Res.* **74,** 19 (1974).
51. A. Dahlström and K. Fuxe, *Acta physiol. scand.* **62,** Suppl. 232, 1 (1964).
52. U. Ungerstedt, *Acta physiol. scand.* Suppl. **367,** 1 (1971).
53. Y. Lapierre, A. Beaudet, N. Demianczuk and L. Descarries, *Brain Res.* **63,** 175 (1973).
54. U. Ungerstedt, *Eur. J. Pharmac.* **5,** 107 (1968).
55. U. Ungerstedt, in *6-Hydroxydopamine and Catecholamine Neurons* (Eds. T. Malmfors and H. Thoenen), p. 101. North-Holland, Amsterdam (1971).
56. L. Descarries and G. Saucier, *Brain Res.* **37,** 310 (1972).
57. U. Ungerstedt, in *Neurosciences Research. Chemical Approaches to Brain Function* (Eds. S. Ehrenpreis and I. J. Kopin) Vol. 5. Academic Press, New York (1973).
58. K. M. Taylor, D. W. Clark, R. Laverty and E. L. Phelan, *Nature, New Biol.* **239,** 247 (1972).
59. L. L. Iversen, *Eur. J. Pharmac.* **10,** 408 (1970).
60. T. Nose, T. Segawa and H. Takagi, *Japan J. Pharmac.* **22,** 867 (1972).
61. Ch. Sachs, in *6-Hydroxydopamine and Catecholamine Neurons* (Eds. T. Malmfors and H. Thoenen), p. 59. North-Holland, Amsterdam (1971).
62. M. Votavova, D. Y. Boullin and E. Costa, *Life Sci.* **10,** 87 (1971).
63. P. U. Angeletti and R. Levi-Montalcini, *Arch. Ital. Biol.* **108,** 213 (1970).
64. T. Malmfors, in *6-Hydroxydopamine and Catecholamine Neurons* (Eds. T. Malmfors and H. Thoenen), p. 353. North-Holland, Amsterdam (1971).
65. Ch. Sachs and G. Jonsson, *J. Neurochem.* **21,** 1517 (1973).
66. F. E. Bloom, S. Algeri, A. Groppetti, A. Revuelta and E. Costa, *Science, N.Y.* **166,** 1284 (1969).
67. L. Laguzzi, F. Petitjéan, Y. F. Pujol and M. Jouvet, *C. r. Soc. Biol., Paris* **165,** 1649 (1971).
68. E. G. Shaskan and S. H. Snyder, *J. Pharmac. exp. Ther.* **175,** 404 (1970).
69. R. E. Heikkila and G. Cohen, *Eur. J. Pharmac.* **21,** 66 (1973).
70. B. R. Cooper, G. R. Breese, L. D. Grant and J. L. Howard, *J. Pharmac. exp. Ther.* **185,** 358 (1973).
71. L. J. Poirier, P. Langelier, A. Roberge, R. Boucher and A. Kitsikis, *J. Neurol. Sci.* **16,** 401 (1972).
72. R. Kostrzewa and D. Jacobowitz, *Eur. J. Pharmac.* **21,** 70 (1973).

73. U. Ungerstedt, in *6-Hydroxydopamine and Catecholamine Neurons* (Eds. T. Malmfors and H. Thoenen), p. 315. North-Holland, Amsterdam (1971).
74. L. Maler, H. C. Fibiger and P. L. McGeer, *Exp. Neurol.* **40,** 505 (1973).
75. T. Hökfelt and U. Ungerstedt, *Brain Res.* **60,** 269 (1973).
76. P. Lidbrink and G. Jonsson, *J. Neurochem.* **22,** 617 (1972).
77. Y. Agid, F. Javoy, J. Glowinski, D. Bouvet and C. Sotelo, *Brain Res.* **58,** 291 (1973).
78. L. L. Butcher and G. K. Hodge, *Proc. Soc. Neuroscience, Third Annual Meeting*, San Diego, Calif., p. 372 (1973).
79. C. Sotelo, F. Javoy, Y. Agid and J. Glowinski, *Brain Res.* **58,** 269 (1973).
80. G. Jonsson, S. Lohmander and Ch. Sachs, *Biochem. Pharmac.* **23,** 2585 (1974).
81. C. E. Hill, G. E. Mark, O. Eränkö, L. Eränkö and G. Burnstock, *Eur. J. Pharmac.* **23,** 162 (1973).