CHEMICAL SYMPATHECTOMY OF THE CAT WITH 6-HYDROXYDOPAMINE

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ABSTRACT


Cats were given 6-hydroxydopamine (6-OH-DA; 2,4,5-trihydroxyphenethylamine) i.v. according to a schedule which had previously been found to produce a selective and almost complete destruction of peripheral adrenergic nerve terminals. After varying intervals, spinal preparations, as well as the isolated medial smooth muscle of the nictitating membrane and the isolated perfused heart, were used to study the 6-OH-DA-induced alterations of the effects of sympathetic nerve stimulation and of the sensitivity to norepinephrine. Twelve to 16 days after 6-OH-DA, the norepinephrine content of heart, spleen, nictitating membrane and iris was below 10% of the normal value. At this time, the effects of sympathetic nerve stimulation on the nictitating membrane and heart were strongly reduced. Surgical denervation was somewhat more effective than 6-OH-DA on the nictitating membrane. After both chemical and surgical sympathectomy, the sensitivity to norepinephrine of the isolated nictitating membrane had increased approximately 100-fold; both procedures seem to have induced the presynaptic as well as the postsynaptic types of supersensitivity. Only a 3-fold increase in the sensitivity to norepinephrine was observed in the isolated heart, and evidence was obtained for the absence of the postsynaptic type of supersensitivity in this organ. The damage to the adrenergic nerve terminals caused by 6-OH-DA was reversible; 3 to 4 weeks after chemical sympathectomy, there was a distinct increase in the norepinephrine content of the organs investigated, and after 14 weeks norepinephrine levels were normal again. The effectiveness of sympathetic nerve stimulation also returned gradually, but more rapidly than the levels of tissue norepinephrine.

A single injection of 6-hydroxydopamine (6-OH-DA) markedly reduces the norepinephrine content in various sympathetically innervated organs of different species for several days or weeks (Porter et al., 1963, 1965; Laverty et al., 1965). In order to explain the long duration of the effect of 6-OH-DA, it was suggested that this amine or possible metabolites of it might replace the physiologic transmitter or that it might irreversibly damage the storage sites for norepinephrine (Porter et al., 1963, 1965; Stone et al., 1964; Laverty et al., 1965).

In recent electron microscopic investigations, Tranzer and Thoenen (1967, 1968) observed a degeneration of adrenergic axon terminals, visible two to three days after treatment with 6-OH-DA. The degeneration selectively concerned the terminal part of the adrenergic neuron; its cell body and other tissue structures, especially cholinergic nerve endings, were morphologically left unaffected by 6-OH-DA. Thoenen and Tranzer (1968a,b) elaborated a treatment schedule for 6-OH-DA that leads to a generalized and virtually complete destruction of adrenergic nerve terminals and proposed the term "chemical sympathectomy."

The present investigation in cats deals with the consequences of chemical sympathectomy as regards the effect of sympathetic nerve stimulation and the sensitivity to norepinephrine. The experiments were performed on spinal cats, on isolated smooth muscles of the nictitating membrane and on isolated hearts of the cat at
various intervals after treatment with 6-OH-DA. On the isolated nictitating membrane, the effectiveness of surgical and chemical sympathectomy was compared.

**Methods.** Cats of either sex, weighing between 1.6 and 2.8 kg, were used.

**Isolated nictitating membrane.** (See Haeusler and Haefely, 1968.) The cats were anesthetized with ether, and spinal preparations were made as described by Burn (1952). The eyeball was removed, together with the orbital fascia, and transferred to a dish filled with ice-cold Tyrode’s solution. After separation from the eyeball, the orbital fascia was spread out, and the medial smooth muscle was freed from the surrounding tissue under a binocular dissecting microscope. Care was taken to preserve the fine sympathetic branch which arises from the infratrochlear nerve and innervates the medial smooth muscle. The cartilage on one side of the isolated medial smooth muscle was fixed to the bottom of a 10-ml organ bath, and the other end of the muscle was connected to a Statham force transducer. The temperature was maintained at 37°C. A slightly modified Tyrode’s solution was used (in millimoles per liter): NaCl, 135; KCl, 3.8; CaCl₂, 2.2; MgSO₄, 0.9; NaH₂PO₄, 0.4; NaHCO₃, 12; glucose, 5.5. The organ bath was gently bubbled with a 95% O₂-5% CO₂ mixture.

A period of about one hour was allowed to elapse before starting the experiment. During this period, the modified Tyrode’s solution was replaced every five minutes. As a rule, the resting tone of the muscle, which was repeatedly readjusted to 2 g, reached steady conditions after 40 to 50 minutes.

The infratrochlear nerve was pulled through shielded bipolar platinum electrodes for stimulation with monophasic rectangular pulses of 0.1-msec duration and supramaximal voltage delivered by a Grass S5 stimulator. “Stimulus number–response curves” were obtained by recording the tension developed when a single shock or trains of 3, 9 and 27 shocks were applied at a stimulation frequency of 1.6/sec. For “frequency–response curves,” the cumulative effect of stimuli with a frequency progressively doubled from 0.2/sec up to 25/sec was determined.

Cumulative dose–response curves for norepinephrine were obtained by adding norepinephrine to the bath fluid. Usually, 1 to 5 minutes after drug addition, the tension developed by the muscle reached a steady level. Each dose of norepinephrine was contained in 0.05 ml. Dilutions were prepared from a stock solution containing norepinephrine in a concentration of 10⁻⁴ M dissolved in 0.02 N HCl. Only one dose-response curve was obtained in any single preparation since a large number of preliminary experiments had shown that, after a full dose-response curve, the sensitivity of the medial smooth muscle toward the same substance decreased somewhat.

**Pretreatment with reserpine.** Cocaine causes a contraction of the normal nictitating membrane which is not observed after pretreatment with reserpine. In experiments with cocaine, cats therefore received an i.p. injection of 3 mg/kg of reserpine 16 hours prior to the preparation of the nictitating membrane.

**Isolated cat heart with sympathetic nerve supply intact.** Isolation, perfusion and sympathetic nerve stimulation of the cat heart were carried out as described earlier (Haeusler et al., 1969b). In all cases atropine sulfate (final concentration, 10⁻⁴ g/ml) was added to the perfusion solution. Drug injections were made into the aortic cannula in the close vicinity of the aortic valves.

** Determination of the ED₅₀ and the slopes of dose-response curves.** All results are expressed as percentages of the maximal development of tension (isolated nictitating membrane) or of the maximal increase in heart rate (isolated cat heart). The horizontal shifts of dose-response curves are measured at the level of the ED₅₀. To facilitate the comparison with the results of Langer and Trendelenburg (1969), we expressed the slope of the dose-response curve in the same way (50/\log ED₅₀ — log ED₅₀). The significance of differences between the mean values of two groups was evaluated by Student’s t test and the range test, which both gave the same results.

**Spinal cats.** Cats were anesthetized with ether and made spinal by section of the spinal cord at the first cervical level and mechanical destruction of the brainstem. The animals were under positive pressure respiration throughout the experiment. A polyethylene catheter was inserted into a femoral artery and connected to a Statham pressure transducer. A femoral vein was cannulated for i.v. injection. The head was fixed in a simplified stereotaxic head holder. Threads were pulled through the cartilaginous margin of the nictitating membranes and connected to a Statham transducer for semi-isometric recording. The resting tension was 4 g. Both cervical sympathetic trunks were cut low in the neck and laid on bipolar platinum electrodes for stimulation. Monophasic rectangular pulses of 0.5-msec duration at supramaximal voltage were delivered from Grass S4 stimulators. Stimulus-number and frequency-response curves were obtained as already described for the isolated nictitating membrane. The main deflection of an electrocardiogram (ECG) lead triggered a cardiostachometer for continuous recording of the heart rate. Arterial blood pressure,
tension of the nictitating membranes and instantaneous heart rate were recorded on a Grass P7 polygraph.

Chemical sympathectomy of cats by 6-OH-DA. For destruction of adrenergic nerve endings, the same schedule of treatment was used as described previously (Thoenen and Tranzer, 1968a,b; Haeusler et al., 1968a). Cats were given two doses of 20 mg/kg of 6-OH-DA hydrobromide i.v. (corresponding to two doses of 14 mg/kg of 6-OH-DA) on the first day, and one week later two doses of 50 mg/kg of 6-OH-DA hydrobromide (corresponding to two doses of 34 mg/kg of 6-OH-DA). 6-OH-DA hydrobromide was dissolved immediately before injection in 0.001 N HCl which was gassed with nitrogen.

Estimation of tissue norepinephrine. The organs were removed from cats anesthetized with pentobarbital and were frozen in petroleum ether at about −80°C, weighed and homogenized in ice-cold 0.4 N HClO4. The norepinephrine values are expressed in terms of micrograms per gram, with the exception of the iris, in which case the values are given in micrograms per organ. The fluorimetric determination of norepinephrine in the medial smooth muscle of the nictitating membrane was carried out following the method of Håggendal (1963) and in the other organs according to Bertler et al. (1958).

The drugs used were: atropine sulfate (C. H. Boehringer und Soehne GmbH., Ingelheim, Germany), cocaine hydrochloride (Ph.H.V.), 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP, Parke, Davis and Company, Detroit, Mich.), l-norepinephrine, tyramine hydrochloride (Fluka AG, Buchs SG, Switzerland), and reserpine (Serpasil, Ciba Pharmaceutical Company, Basel, Switzerland). 2,4,5-Trihydroxyphenethylamine hydrobromide (6-hydroxydopamine) was synthesized by Dr. A. Langemann of the Chemical Research Department of F. Hoffmann-La Roche & Co. Ltd., Basle, Switzerland.

Concentrations or doses of norepinephrine and tyramine are expressed as log molar concentrations or as log grams, respectively. Doses of norepinephrine and tyramine refer to the free base. Norepinephrine was diluted from a stock solution with saline containing ascorbic acid (1 mg/ml).

RESULTS. ISOLATED NICTITATING MEMBRANE. Twelve to 16 days after the first injection of 6-OH-DA, suppression of the sympathetic nerve with frequencies of up to 6.4 shocks/sec produced no contraction of the isolated nictitating membrane. At a stimulation rate of 12 or 25 shocks/sec, a small response occurred (fig. 1A). The isolated nictitating membrane of cats whose superior cervical ganglia had been extirpated 12 to 16 days prior to the experiment did not respond to sympathetic nerve stimulation at any frequency (fig. 1A). Accordingly, the norepinephrine content of smooth muscles of nictitating membranes denervated surgically was reduced virtually to zero, whereas that of chemically sympathectomized nictitating membranes was on the average 8% of the control value (fig. 1C). Similar results were obtained in the iris, in which the corresponding norepinephrine values were zero and 3%, respectively (fig. 2B).

The medial smooth muscles of denervated nictitating membranes were supersensitive to norepinephrine. The ED50 of norepinephrine in surgically denervated membranes was shifted to the left by a factor of 96 as compared to the controls, and in chemically sympathectomized membranes by a factor of 71 (fig. 3; table 1). A precise comparison is, however, not possible since the slope of the dose-response curves of the denervated membranes was significantly smaller than that of the curve obtained in normal membranes (table 1).

Dose-response curves for norepinephrine in the presence of cocaine (10−4 M) were determined in innervated nictitating membranes from reserpinized-pretreated cats since this pretreatment abolishes the contraction caused by cocaine. As shown in figure 3 and table 1, cocaine caused the dose-response curve to shift to the left by a factor of 15. The dose-response curve determined in the presence of cocaine was significantly flatter than without cocaine but significantly steeper than those of denervated membranes (table 1). There was no statistically significant difference between any groups in the maximal tension produced by a supramaximal concentration of norepinephrine.

ISOLATED CAT HEART. Twelve to 16 days after the first injection of 6-OH-DA, the effect of electrical sympathetic nerve stimulation on the increase in heart rate was abolished when low frequencies of stimulation were used and strongly reduced with frequencies between 6.4 and 25 shocks/sec (fig. 4A). The results were very similar to those obtained with isolated nictitating membranes. Sympathetic nerve stimulation (at any frequency) never produced a positive inotropic effect. Basal heart rate after
Fig. 1. The effect of surgical and chemical adrenergic denervation on the response of the isolated medial smooth muscle of the nictitating membrane to sympathetic nerve stimulation (A) and to norepinephrine (B) and on its content of norepinephrine (C). ●, controls; △, 2 weeks after surgical denervation; ○, 2 weeks, □, 4 weeks and ■, 8 weeks after the first injection of 6-OH-DA. Shown are the mean values (±S.E. as vertical bars).

A. Response to supramaximal stimulation of the infratrochlear nerve at frequencies increasing from 0.2/sec to 25/sec and to varying numbers of stimuli at 1.6/sec. Controls (n = 18), 2 weeks after surgical denervation (n = 6), and 2 weeks (n = 6) and 4 weeks (n = 4) after the first injection of 6-OH-DA.

B. Dose-response curves for norepinephrine. For number of experiments, consult table 1.

C. Norepinephrine content at the indicated intervals after the first injection of 6-OH-DA (n = 6; 14 weeks, n = 2). △, norepinephrine content after surgical denervation (n = 5).

Fig. 2. Norepinephrine content of spleen (A) and iris (B) at the indicated intervals after the first injection of 6-OH-DA (n = 6; 14 weeks, n = 2). In B, △ marks the norepinephrine content after surgical denervation (n = 5). Shown are the mean values (±S.E. as vertical bars).

A. Log M concentration of norepinephrine (ng/g) in the spleen at the indicated intervals after the first injection of 6-OH-DA (n = 6).

B. NE-content (% of control) of spleen at the indicated intervals after the first injection of 6-OH-DA (n = 6).

C. Norepinephrine content at the indicated intervals after the first injection of 6-OH-DA (n = 6; 14 weeks, n = 2). △, norepinephrine content after surgical denervation (n = 5).
chemical sympathectomy was 136.4 ± 7.8 (S.E.) beats/min and did not differ significantly from that of controls (127.1 ± 6.3). The norepinephrine content of the hearts was at an average 3% of the normal value (fig. 4C).

In contrast to the isolated nictitating membranes, the dose-response curve (measured parameter = increase in heart rate) for norepinephrine on the isolated cat heart was shifted to the left by a factor of only 3.5 after chemical sympathectomy (fig. 4B; table 2). The same shift of the dose-response curve for norepinephrine was observed in some preliminary experiments on innervated hearts when cocaine or desipramine was added to the perfusion liquid. Chemical sympathectomy did not change the slope of dose-response curves for norepinephrine. Twelve to 16 days after chemical sympathectomy, the effect of tyramine on heart rate and force of contraction was practically abolished (fig. 4B; table 2). The slope of the dose-response curve for tyramine in innervated hearts was the same as for norepinephrine.

**Spinal Cat.** In cats treated with 6-OH-DA, no miosis nor relaxation of nictitating membranes was observed 12 to 14 days after the first injection of 6-OH-DA. Their general behavior did not differ from that of control cats. Results obtained on the nictitating membrane and the cardiovascular system are summarized in figure 5.

**Nictitating membrane.** When the cats were set up as a spinal preparation, the responses of their nictitating membranes to supramaximal

![Image of graph](https://via.placeholder.com/150)

**Fig. 3.** Responses of the isolated medial smooth muscle of the nictitating membrane to norepinephrine. Symbols are the same as in figure 1. A, dose-response curve for norepinephrine of normal membranes in the presence of cocaine (10⁻⁶ M). Shown are the mean values (±S.E. as vertical bars). For number of experiments, consult table 1.

**TABLE 1**

<table>
<thead>
<tr>
<th>Agents and Procedures*</th>
<th>n°</th>
<th>ED50 (Mean ± S.E.)</th>
<th>Relative Sensitivity to Norepinephrine</th>
<th>Slope (Mean ± S.E.)*</th>
<th>Maximum Contraction (Mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>6</td>
<td>5.082 ± 0.058</td>
<td>1</td>
<td>46.7 ± 2.0</td>
<td>15.5 ± 0.5</td>
</tr>
<tr>
<td>10⁻⁴ M cocaine + 3 mg/kg reserpine</td>
<td>7</td>
<td>6.248 ± 0.002²</td>
<td>15</td>
<td>31.7 ± 1.1⁴</td>
<td>14.5 ± 1.1</td>
</tr>
<tr>
<td>2 weeks after surgical denervation</td>
<td>6</td>
<td>7.064 ± 0.138⁴</td>
<td>96</td>
<td>19.2 ± 1.1²</td>
<td>17.5 ± 1.9</td>
</tr>
<tr>
<td>Time after first injection of 6-OH-DA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>6</td>
<td>6.936 ± 0.060⁴</td>
<td>71</td>
<td>21.2 ± 1.6²</td>
<td>16.7 ± 1.0</td>
</tr>
<tr>
<td>3-4 weeks</td>
<td>4</td>
<td>6.668 ± 0.285⁴</td>
<td>38</td>
<td>22.7 ± 2.2⁴</td>
<td>14.6 ± 1.1</td>
</tr>
<tr>
<td>8-9 weeks</td>
<td>5</td>
<td>5.190 ± 0.040</td>
<td>1.3</td>
<td>43.8 ± 2.1</td>
<td>16.1 ± 0.7</td>
</tr>
</tbody>
</table>

* See text for details.

* Number of experiments.

* Values significantly different from the normal (P < .001).

* Value significantly different from each of the other values in the same column (P < .01).
preganglionic stimuli, either continuously applied at frequencies between 0.2/sec and 25/sec or varying in number between 1 and 27 at 1.6/sec, were greatly reduced. The effect of repetitive stimulation at different rates was, on the average, reduced to 27% ± 1.8 (S.E.) of the controls, and the effect of varying trains of stimuli at a fixed rate of 1.6/sec was reduced to 25% ± 3.5 (S.E.) of the control values. The nictitating membranes after 6-OH-DA treatment were about 100 times more sensitive to i.v. injected norepinephrine than the controls. Tyramine was somewhat more effective at the lower dose than in the controls, but its effect was greatly reduced at the highest dose (3 mg/kg) tested (to 32% of controls). The sensitivity to DMPP was increased 30-fold in 6-OH-DA-treated cats.

**Pressor effect.** The pressor effect of norepinephrine was markedly increased. The shift of the dose-response curve was about 10-fold as compared to the controls. The dose-response curve for tyramine was greatly depressed, 3 mg/kg eliciting only 35% of the blood pressure elevation of control animals. The shift of the dose-response curve to the left for DMPP was about 5-fold.

**Cardiac pacemaker.** The cardiac pacemaker appeared to be about 10 times more sensitive to norepinephrine in 6-OH-DA-treated animals. The maximal increase in heart rate obtainable with norepinephrine was apparently depressed. This depression was not real but due to the fact that the basal heart rate of 6-OH-DA-treated spinal cats was considerably higher than in untreated controls (206 ± 14 and 148 ± 5.4 beats/min, respectively). In the doses used, tyramine was only slightly active on the cardiac pacemaker of 6-OH-DA-treated spinal cats, and the maximal positive chronotropic effect of norepinephrine. Shown are the mean values (±S.E. as vertical bars).

A. Response to supramaximal stimulation of the cardiac sympathetic nerves at frequencies increasing from 0.2/sec to 25/sec and with varying numbers of stimuli at 1.6 sec. ●, controls (n = 21); ○, 2 weeks (n = 10); □, 4 weeks (n = 8) and ■, 8 weeks (n = 8) after the first injection of 6-OH-DA.

B. Dose-response curves for norepinephrine (circles): ●, controls (n = 10); ○, 2 weeks after the first injection of 6-OH-DA (n = 4). Dose-response curves for tyramine (squares): □, controls (n = 8); ■, 2 weeks after the first injection of 6-OH-DA (n = 4).

C. Norepinephrine content at the indicated intervals after the first injection of 6-OH-DA (n = 6; 14 weeks, n = 2).

**Fig. 4.** The effect of chemical sympathectomy on the response of the isolated perfused heart to sympathetic nerve stimulation (A) and to norepinephrine and tyramine (B) and on its content of norepinephrine. Shown are the mean values (±S.E. as vertical bars).
TABLE 2

The effect of chemical sympathectomy on the chronotropic response of the isolated heart to norepinephrine and tyramine

<table>
<thead>
<tr>
<th>Agents and Procedures*</th>
<th>EDSO (Mean ± S.E.)</th>
<th>Relative Sensitivity to Norepinephrine</th>
<th>Slope (Mean ± S.E.)</th>
<th>Maximum Increase in Heart Rate (Mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n*</td>
<td>-log 2</td>
<td>50/(log EDSO - log EDS0)</td>
<td>beats/min</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>6.973 ± 0.070</td>
<td>1</td>
<td>59.0 ± 4.2</td>
</tr>
<tr>
<td>12-16 days after first injection of 6-OH-DA</td>
<td>4</td>
<td>7.522 ± 0.050*</td>
<td>3.5</td>
<td>59.7 ± 3.6</td>
</tr>
<tr>
<td>Tyramine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>6</td>
<td>4.990 ± 0.065</td>
<td>0.01</td>
<td>59.2 ± 3.3</td>
</tr>
<tr>
<td>12-16 days after first injection of 6-OH-DA</td>
<td>4</td>
<td></td>
<td></td>
<td>4.0 ± 1.8*</td>
</tr>
</tbody>
</table>

* See text for details.
*b Number of experiments.
*c See text for calculation.
*d Values significantly different from the normal (P < .001).

tyramine was about 40% of that obtainable with norepinephrine. The dose-response curve for DMPP seemed unimpaired by 6-OH-DA except that the maximal increase of the heart rate above the resting value was apparently depressed in a similar way as for norepinephrine.

RECOVERY AFTER CHEMICAL SYMPATHETOMY. As the time interval after chemical sympathectomy increased, the norepinephrine content of heart, spleen, nictitating membrane and iris slowly rose again. Three to four weeks after the first injection of 6-OH-DA, the norepinephrine content varied between 14 and 22% of the normal value, depending on the organ, and after eight to nine weeks, between 30 and 85% (figs. 1C; 2, A and B; 4C). Two cats, which were examined 14 weeks after the chemical sympathectomy, had an essentially normal norepinephrine content of heart, spleen, nictitating membrane and iris.

In the isolated nictitating membrane, the effect of sympathetic nerve stimulation was completely restored three to four weeks after treatment with 6-OH-DA, whereas the norepinephrine content of the medial smooth muscle was only 22% of that of the controls (fig. 1, A and C). At this time the sensitivity of the medial smooth muscle to norepinephrine was still clearly increased, and the slope of the dose-response curve for norepinephrine was decreased as compared to the controls (fig. 1B; table 1).

Eight to nine weeks after the chemical sympathectomy, when the norepinephrine content was 69% of that of the controls, the sensitivity of the isolated nictitating membrane to norepinephrine had returned to normal. The slope of the dose-response curve for norepinephrine was then identical with that of the controls (fig. 1, B and C; table 1).

The isolated heart behaved in a different manner. Three to four weeks after the first injection of 6-OH-DA, the effect of sympathetic nerve stimulation on the heart rate was still reduced, and even after eight to nine weeks, the response to high frequencies of stimulation was slightly decreased (fig. 4A). The corresponding values of the content of norepinephrine in the heart were 16% and 30%, respectively, of the normal (fig. 4C).

DISCUSSION. In this investigation functional alterations occurring in certain organs of the cat after chemical sympathectomy with 6-OH-DA were studied and compared with the changes in the norepinephrine content of the tissue. The schedule of 6-OH-DA-treatment used was elaborated by Thoenen and Tranzer (1968a,b) and leads to the greatest possible reduction of tissue norepinephrine. The results reported here were obtained 12 to 16 days after the first of four injections of 6-OH-DA. At that time the tissue content of norepinephrine is still at its lowest level (Thoenen and Tranzer, 1968b), and
denervation supersensitivity could be expected to be fully developed (Langer et al., 1967). The recovery from chemical sympathectomy was followed up for three months.

A series of experiments was carried out on the spinal cat, but the experiments on the isolated nictitating membrane and the perfused heart seem to give a more accurate indication of noradrenaline sensitivity in the respective organs. Both in situ and in vitro, the response of the nictitating membrane and heart to noradrenaline depends on amine-receptor combination at the effector cells and on a chain of events induced by the receptor activation (so-called postsynaptic events) as well as on the inactivation mainly by uptake (so-called presynaptic events). In the spinal cat, however, the response to i.v. injected noradrenaline is additionally influenced by presynaptic events on the way from the site of injection to the target organ.

Nictitating membrane. Twelve to 16 days after the first injection of 6-OH-DA, the response to sympathetic nerve stimulation of the nictitating membrane in situ as well as in vitro was greatly reduced but not completely abolished, as it was after surgical sympathectomy by removing the superior cervical ganglion. In the nictitating membrane, which is easy to denervate surgically, chemical sympathectomy there-
fore seems to be slightly less effective than surgical denervation. This is also reflected by the virtual disappearance of norepinephrine from the tissue after surgical denervation in comparison with a mean residual norepinephrine content of 8% after chemical sympathectomy. The same finding holds true for the iris.

It is not clear why neurally evoked contractions of the nictitating membrane were more depressed in vitro than in vivo after 6-OH-DA. The simplest explanation would be that, for some unknown reason, the same treatment did not lead to the same degree of degeneration in the group of cats used for spinal preparations. Even more speculative is the assumption that some of the nerve terminals which did not degenerate after 6-OH-DA had at least part of their vesicles destroyed (or functionally impaired). These fibers could still release a certain quantity of norepinephrine upon stimulation in vivo but only a smaller amount under the less optimal conditions in vitro (e.g., absence in the incubation medium of norepinephrine precursors and possibly of other unknown factors facilitating synthesis and storage of norepinephrine).

After both chemical and surgical sympathectomy, the nictitating membrane in vivo and in vitro had developed an appreciable supersensitivity to norepinephrine. Sensitivity after surgical sympathectomy was slightly greater than after treatment with 6-OH-DA, but the difference was not statistically significant. On the isolated medial smooth muscle of the denervated nictitating membrane, the ED50 of norepinephrine was shifted to the left by approximately 2 log units as compared with innervated organs. Cocaine (10^-4 M) in innervated nictitating membranes produced a shift to the left by a factor of 15. Although cocaine might not have blocked the neuronal uptake of norepinephrine completely, this difference of sensitivity between cocaine-treated and sympathectomized membranes seems to indicate, but does not conclusively prove, that postsynaptic supersensitivity had additionally developed after chemical and surgical sympathectomy.

Tsai et al. (1968) could not demonstrate the development of postsynaptic supersensitivity to norepinephrine in the isolated nictitating membrane preparation after surgical sympathetic denervation. They found that after denervation, as well as after blocking the uptake by cocaine in innervated membranes, the dose-response curve for norepinephrine was shifted to the left by the same value. Their results are in contrast to ours and to those obtained by Langer et al. (1967) in the spinal cat after surgical sympathetic denervation.

In denervated nictitating membranes and in membranes treated with cocaine, the dose-response curves for norepinephrine were flatter than in control membranes. This confirms the findings of Langer and Trendelenburg (1969); they demonstrated that, in the nictitating membrane, the increasing saturation of the neuronal uptake of norepinephrine disproportionately changes the concentration of the amine at the receptors, as compared with its concentration in the bath fluid, and consequently alters the slope of the dose-response curve. In our experiments, the slopes of the dose-response curves in denervated membranes and the slopes of the dose-response curves in membranes treated with cocaine were different from each other. This may be an indication that cocaine (10^-4 M), as already suggested, did not completely block the uptake of norepinephrine.

The return of norepinephrine after chemical sympathectomy was rather rapid, a finding not too surprising since ultramorphologic studies had revealed degeneration of only the most terminal parts of adrenergic nerve axons (Tranzer and Thoenen, 1967, 1968). The norepinephrine content of the nictitating membrane started to increase by the third to fourth week after the beginning of the treatment with 6-OH-DA and reached normal values after three months, at which time Tranzer and Thoenen (1968) could no longer detect ultrastructural signs of degeneration. Functionally, recovery was even more rapid. Three to four weeks after treatment, the responses of the medial smooth muscle to sympathetic nerve stimulation were of normal size, although the norepinephrine content was only one-quarter of the normal value. Obviously, the still marked supersensitivity to norepinephrine, probably of the postsynaptic type, could compensate for the reduced amounts of transmitter released by nerve stimulation. Eight to nine weeks after treatment, the sensitivity to norepinephrine reached normal values, and the norepinephrine content was 69% of the normal.

Dose-response curves for norepinephrine were equally flat two weeks as well as four weeks
after chemical sympathectomy, whereas the slope had increased to the control value after eight to nine weeks. It would have been especially interesting to know whether the partial recovery of norepinephrine content after four weeks was paralleled by a similar degree of recovery of uptake capacity. Identical slopes two and four weeks after sympathectomy suggest at first sight the absence of neuronal uptake after four weeks in spite of a norepinephrine content of 22% of the controls, thus indicating a discrepancy between the rate of recovery of storage sites and that of uptake mechanism. However, the sensitivity of the nictitating membrane, which was at that time still increased 38-fold, shifted the effective concentrations of norepinephrine into that range at which uptake seems to be a linear function of the concentration of norepinephrine in the bath fluid (Langer and Trendelenburg, 1969). Therefore, it is impossible to decide whether at this stage of the recovery the low slope observed is due to absence or to linearity of uptake.

Heart. Treatment with 6-OH-DA greatly reduced the positive chronotropic effect of sympathetic nerve stimulation and abolished the positive inotropic effect in the isolated heart. In contrast to the nictitating membrane, the heart became only 3.5 times more sensitive to norepinephrine. This denervation supersensitivity was of the same order of magnitude as the maximal supersensitivity obtainable with cocaine or desipramine. This seems to indicate that in the heart denervation supersensitivity after 6-OH-DA is of presynaptic origin and that postsynaptic supersensitivity did not occur, as was probably the case in the nictitating membrane. Whether the different type of receptors (beta adrenergic in the heart, alpha adrenergic in the nictitating membrane) or the different type of muscle involved is responsible for the different behavior is open to speculation. It is interesting that Dempsey and Cooper (1968) found no indication for a postsynaptic type of norepinephrine supersensitivity in the cat heart after medastinal neural ablation. Thus, neither chemical nor surgical cardiac sympathectomy seems to induce postsynaptic supersensitivity to norepinephrine in the heart, which again demonstrates the qualitative similarity of the changes produced by these two procedures. In the present series of experiments, the norepinephrine content of the heart was 3% after chemical sympathectomy, as compared to 10 to 20% found by Hertting and Schiefthaler (1964) after bilateral extirpation of the stellate ganglia and the complete loss of norepinephrine observed by Cooper (1966) after cardiac autotransplantation.

Although uptake could be expected to be virtually abolished after chemical sympathectomy, the dose-response curves for norepinephrine had identical slopes in innervated and denervated hearts. This is consistent with the finding of a relatively small presynaptic supersensitivity of the heart to norepinephrine after denervation and hence with a relatively low uptake capacity for norepinephrine as compared to the nictitating membrane. Moreover, saturation of uptake may start only at concentrations of norepinephrine exceeding those used to obtain a maximum effect in innervated hearts.

Functional recovery from chemical sympathectomy was slower in the heart than in the nictitating membrane. Whereas in the latter organ the effect of sympathetic nerve stimulation was completely restored three to four weeks after the end of the treatment with 6-OH-DA, it was still slightly impaired in the heart after eight to nine weeks. One reason for this is the absence of a postsynaptic supersensitivity to norepinephrine in the heart after chemical sympathectomy. Another reason may be that the chemical sympathectomy was more effective in the heart than in the nictitating membrane, as judged from the norepinephrine content. Furthermore, during the phase of recovery, the amount of cardiac norepinephrine was always lower than that of the nictitating membrane.

Cardiovascular responses in the spinal cat. The pressor response to norepinephrine was increased 10 to 30 times two weeks after chemical sympathectomy. This value is somewhat greater than that found in the heart, and it is tempting to speculate that this difference is due to the supersensitivity of the various vascular beds. The pressor response is, however, too complex a parameter to permit such a conclusion. Therefore, the question whether or not 6-OH-DA achieves an efficient denervation of blood vessels must be left open. The pressor effect of DMPP was also increased after 6-OH-DA although less than that of norepinephrine. DMPP is a potent stimulant of the adrenal medulla, and its pressor effect may to a great extent be due to released epinephrine. Supersensitivity to norepinephrine and epinephrine possibly coupled with increased
activity of the adrenal medulla may explain the absence of arterial hypotension in cats sympathectomized with 6-OH-DA (M. Gerold, personal communication). The tyramine pressor effect was markedly reduced. Tyramine had virtually lost its positive chronotropic effect and, in doses above 1 mg/kg, even decreased the heart rate in two out of seven animals. A negative chronotropic effect of sympathomimetic amines was described by Trendelenburg et al. (1963) in isolated guinea-pig atria. The interpretation of the altered chronotropic effect of norepinephrine and DMPP is rendered difficult by the higher resting heart rate of the spinal preparations of 6-OH-DA-treated cats.

Our experiments suggest a qualitatively similar effect of chemical and surgical sympathectomy in the heart and nictitating membrane of the cat. Quantitative differences, however, exist between these two methods. Heart, spleen and iris were most affected by 6-OH-DA, as judged from their norepinephrine content, whereas the nictitating membrane was the least affected of the organs studied. Differences in the sensitivity of organs to 6-OH-DA had already been found by Thoenen and Tranzer (1968b) in the rat, in which the norepinephrine content fell to 5 to 8% of the normal value in the spleen and heart, but to 19% only in the vas deferens. The length of the adrenergic fibers may be one of the factors influencing the effectiveness of chemical denervation. Differences in the blood supply of the various organs may be another factor. Finally, adrenergic nerve terminals in various organs might differ in their rate of amine uptake; uptake of 6-OH-DA, however, is a prerequisite for its action (Malmfors and Sachs, 1968).

Conclusions. Chemical sympathectomy with 6-OH-DA seems to be a useful means of selectively destroying adrenergic nerves either for studies of generalized sympathectomy or for denervation of those organs which can be surgically sympathectomized only with great technical difficulty. The superiority over immunosympathectomy is evident. The effect of 6-OH-DA lasts long enough to permit studying alterations produced by chronic sympathetic denervation, and its reversibility (starting three to four weeks after 6-OH-DA treatment and completed three months later) makes it possible to investigate the process of adrenergic reinnervation from the morphologic, biochemical and physiologic aspects. When a selective destruction of adrenergic nerve terminals is important, chemical sympathectomy is superior to surgical sympathectomy, as the latter also interrupts cholinergic fibers present to widely varying degrees in the so-called sympathetic nerves.

REFERENCES


CHEMICAL SYMPATHECTOMY


