Increased Dopamine Efflux from Striatal Slices During Development and After Nigrostriatal Bundle Damage

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Dopaminergic control over striatal targets appears to be retained in rats sustaining lesions of the nigrostriatal dopamine (DA) system as long as 5–10% of that projection remains. Similarly, during postnatal development, dopaminergic control over striatal neurons matures well before the innervation of striatum by the nigrostriatal bundle is attained. These observations suggest that enhanced efficacy of dopaminergic transmission may compensate for hypoinnervation of striatum after lesions or during development. To examine this hypothesis, striatal slices were superfused with Krebs bicarbonate buffer and effluent was collected and analyzed for endogenous DA. Electrical field stimulation (2 Hz) continuously delivered to slices prepared from intact adult rats increased DA efflux to 3–5 times the prestimulation rate within 10 min. Efflux then fell to approximately twice the basal rate over the next 20 min. DA efflux was also examined using slices prepared from adult animals given 6-hydroxydopamine 2–3 weeks earlier, and from 7–10-d-old rat pups. In each group, striatal DA levels were 10–40% of adult control values. Nevertheless, stimulated DA efflux from these slices attained the same rate as that observed with intact, adult slices. Thus, fractional DA efflux from these slices was several times higher than the control rate by the end of the stimulation period. This increased DA efflux appeared to be a consequence of both increased release and decreased reuptake of DA, as the fractional DA efflux from control striatal slices could not be increased to the rate seen in hypoinnervated slices using nomifensine (10 μ M), an inhibitor of DA efflux. Moreover, increased DA biosynthesis was indicated by the maintenance of DA stores in the hypoinnervated slices during stimulation. Thus, increased DA release, decreased reuptake, and increased synthesis may serve to compensate for hypoinnervation after injury or during development.

The dopamine (DA)-containing neurons of the nigrostriatal bundle have significant influences on striatal function, as measured at both the cellular and behavioral levels (Ungerstedt, 1971; Zigmond and Stricker, 1973; Marshall et al., 1974; Agid

et al., 1975; Rommelspacher and Kuhar, 1975; Schultz and Ungerstedt 1978; and others). However, these influences appear to be relatively independent of the density of innervation. Thus, dopaminergic influences mature well before the nigrostriatal bundle is fully developed (Lal and Sourkes, 1973; Baez et al., 1976; Coyle and Campochiaro, 1976). Moreover, in adult animals it is possible to destroy up to 90% of the bundle without permanent disruption of either cellular function or behavior (Zigmond and Stricker, 1973; Creese and Snyder, 1979; MacKenzie et al., 1985; Orr et al., 1986). These latter results cannot be attributed to alterations in the number of postsynaptic receptors, since neonatal rats do not have more DA receptors than adults do (Pardo et al., 1977), and, after DA-depleting lesions in adult animals, postsynaptic supersensitivity occurs only after the loss of at least 90% of striatal DA content (Creese and Snyder, 1979; Zigmond and Stricker, 1980). Instead, there is considerable evidence that, after brain damage in adult rats, the synthesis and turnover of DA within the existing dopaminergic terminals are increased relative to the rates in intact control animals (Agid et al., 1973; Hefti et al., 1980; Zigmond et al., 1984). The increased turnover that occurs after DA depletions of 50–60% or more may reflect an increase in the capacity of these terminals to release DA and thereby compensate for the loss.

The use of brain slices provides a useful experimental approach with which to examine this hypothesis. Such a preparation retains several properties of intact brain tissue, including the capacity for calcium-dependent transmitter release (Raiteri et al., 1984). Slices from striatum contain axon terminals of the dopaminergic nigrostriatal bundle, as well as of other neuronal and non-neuronal elements. Although the dopaminergic terminals are devoid of the spontaneous action potentials normally generated in cell bodies located in substantia nigra, once depolarized they release DA in a calcium- and frequency-dependent manner and thereby affect other elements within the slice (Farnebo and Hamberger, 1971; Starke et al., 1978; Cubeddu and Hoffman, 1983). In this report we have used striatal tissue slices to study depolarization-induced DA efflux in 2 groups of animals: neonatal rats with incomplete dopaminergic innervation of striatum, and adult rats after partial injury of nigrostriatal bundle caused by pretreatment with the neurotoxin 6-hydroxydopamine (6-HDA). Our findings suggest that, in both groups, there is an elevated capacity for DA release as well as a decreased rate of DA reuptake, leading to an increase in transmitter availability in extracellular fluid.

Materials and Methods

Materials. Dopamine HCl, Sephadex G-25, 6-HDA hydrobromide, pargyline and BSA (fraction V) were obtained from Sigma Chemical

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Co., St. Louis, MO. 6-Methyl-5, 6, 7, 8-tetrahydropterin HCl (6MPH₄) was obtained from Calbiochem-Behring, La Jolla, CA, and ³H-dopamine-[ethyl-1-³H(N)] was obtained from New England Nuclear, Boston, MA. Desipramine was the gift of Merrell National Labs, Cincinnati, OH, and nomifensine maleate the gift of Hoechst-Roussel Pharmaceuticals, Somerville, NJ. All remaining reagents were purchased from Fisher Scientific, Pittsburgh, PA, and were of the highest obtainable purity.

Animals. Male Sprague-Dawley rats were used in all experiments. Adult rats weighing 200–250 gm were obtained from Zivic-Miller Laboratories (Allison Park, PA), whereas neonatal rats derived from parents of the same strain were raised in litters of 8–10 in our colony. Adult animals were allowed free access to Purina Rodent Laboratory Chow (Ralston Purina, St. Louis, MO) and tap water, and were housed in a temperature-controlled room (23–25°C) in which illumination was provided from 8 A.M. to 8 P.M.

Adult rats were anesthetized with ether, and then 6-HDA (150–250 µg free base) was administered into the lateral cerebroventricles (Noble et al., 1967). Thirty minutes earlier, the animals had been treated with desipramine (25 mg/kg, i.p.) to block the effects of the neurotoxin on noradrenergic terminals, and with pargyline (40 mg/kg, i.p.) to inhibit monoamine oxidase and thereby enhance the neurotoxicity of 6-HDA (Breese and Traylor, 1970). Control animals either received desipramine, pargyline, and the 6-HDA vehicle (20 µl of a 0.9% NaCl solution containing 0.1% ascorbic acid) ($n = 7$) or no treatment ($n = 3$). Because there were no differences between the 2 control groups, the resulting data have been combined for presentation.

DA release from striatal slices. All animals were killed by decapitation. Neonatal rats were killed 7–10 d postpartum, whereas adult rats were killed 3–4 weeks after 6-HDA treatment. Brains were removed rapidly, placed on ice, and the area containing corpus striatum was dissected out bilaterally. Coronal slices (350 µm; approximately 0.4 ng protein) were prepared using a McIlwain tissue chopper. Three slices, one each from the rostral, middle, and caudal portions of striatum, were used for the estimation of tissue DA and protein contents before superfusion. Eight additional slices were placed into a superfusion chamber (250 µl capacity) equipped with 2 platinum gauze electrodes. Slices were separated from one another with layers of Sephadex G-25 resin (course, 30–100 µm) and from the electrodes with nylon mesh (50 µm pore size). Both the small chamber volume and the Sephadex significantly improved our capacity to detect endogenous DA efflux.

Tissue was superfused at a rate of 100 µl/min with 37°C Krebs bicarbonate medium containing 117 mM NaCl, 4.7 mM KCl, 1.2 mM MgCl₂, 1.25 mM CaCl₂, 1.2 mM NaH₂PO₄, 25 mM NaHCO₃, 0.015% ascorbic acid, 11.5 mM dextrose, and 10 µM tyrosine, and was constantly bubbled with gas containing 5% CO₂ and 95% O₂. A relatively low rate of superfusion was used in order to permit equilibration between extracellular fluid and superfusing medium, and to allow the normal processes of diffusion and reuptake to take place. Five minute fractions (500 µl) were collected in tubes containing 50 µl of 1.1 N perchloric acid and 2.2 mM NaHSO₃ for the subsequent analysis of endogenous DA.

After superfusion for 25 min, slices were depolarized. Field depolarization, rather than KCl, was chosen for this purpose because of its more physiological nature, the ease with which it can be varied, and preliminary findings that KCl often produced a depletion of tissue DA (Keller et al., 1983a). A bipolar current pulse was used to minimize interference from ions generated by the stimulation process. Each current pulse pair consisted of a 20 mA, 2 msec square pulse followed 0.5 msec later by a 2 msec pulse of the opposite sign. Unless otherwise noted, pulse pairs were delivered at 2 Hz, a frequency that is at the low end of the physiological range (Grace and Bunney, 1984). Stimuli were generated by means of a Grass S-88 stimulator in conjunction with a Grass SIU-5 stimulus isolation unit and a Grass CCU-1 constant current unit, and were monitored across a 1000 Ω resistor placed in series with the electrode by a Tektronix D12 oscilloscope. At the end of the experiment, slices were recovered from the chamber and their protein and DA contents were measured. The amount of DA released into superfusion medium was expressed in nanograms of DA per milligram tissue protein per 5 min fraction. In addition, the fractional rate of DA efflux was determined by expressing the amount of DA released as a percentage of the DA present in slices prior to superfusion. Stimulation-induced overflow was calculated by subtracting fractional release during the last 5 min before the onset of stimulation from that observed during 5 min periods of stimulation.

DA concentration. The DA concentration in samples of tissue and

superfusate was measured by high-performance liquid chromatography with electrochemical detection. Tissue slices were homogenized in 0.1 N HClO₄ containing 0.2 mM NaHSO₃ and centrifuged at 40,000 × *g* for 15 min. The resulting pellets were used to measure protein content. The supernatants, as well as fractions collected in the superfusion experiments, were then purified by alumina extraction and DA was measured as described previously (Keller et al., 1976; Zigmond et al., 1984). All samples yielded distinct peaks that were well above the background signal. Values were corrected for recovery using 3,4-dihydroxybenzylamine as an internal standard. Tissue values were expressed as nanograms of DA per milligram of protein.

High-affinity DA uptake. Initial rates of high-affinity DA uptake were measured *in vitro* using minor modifications of previous methods (Stachowiak et al., 1986). A fraction (P₂; Gray and Whittaker, 1962) containing synaptosomes, as well as mitochondria and membrane fragments, was prepared and preincubated for 5 min at 37°C with Krebs medium and 20 µM pargyline. ³H-DA-[ethyl-1-³H(N)] (44.4 Ci/mmol) was then added, yielding a final DA concentration of 10 nM, and the incubation was continued. Five minutes later, tissue was separated by filtration. The filters were washed with ice-cold incubation buffer containing 5 mM DA, dried, placed into scintillation vials containing 1 ml of 0.2 M HClO₄, and quantified by liquid-scintillation spectroscopy. Saturable high-affinity uptake (the difference between total tissue retention of tritium and tritium uptake in the presence of 0.1 mM unlabeled DA) was temperature-dependent and inhibited by 10 µM nomifensine, but it was not affected either by 0.5 µM norepinephrine (NE) or 0.5 µM 5-HT. All samples were run in triplicate. Saturable uptake was proportional to time for a minimum of 5 min and to protein concentration within a range of at least 3–100 µg/sample. Approximately 50,000 and 5000 total cpm were obtained in samples from control and 6-HDA-lesioned rats, respectively. Nonsaturable uptake yielded about 2000 cpm in both groups of rats. Final values were expressed as picomoles of ³H-DA per minute per nanogram of protein.

Tyrosine hydroxylase activity. The soluble tyrosine hydroxylase (TH) activity of crude homogenates was measured by a coupled-decarboxylase method (Waymire et al., 1971; Zigmond et al., 1984). Tissues were homogenized in 50 mM Tris-HCl buffer and a portion of the homogenate was used to measure DA content. The remaining homogenate was centrifuged at 40,000 × *g* for 30 min and the supernatant was passed through a 5 × 60 mm Sephadex G-25 column (50–150 µm particle size; Sigma). TH activity in the supernatant was measured in a 50 mM Tris-acetate buffer (pH 5.8) containing 75 µM L-¹⁴C tyrosine (54 mCi/mmol; New England Nuclear) and 3 mM 6MPH₄. Samples were run in triplicate. Approximately 10,000 and 2000 total cpm were obtained from samples from control and 6-HDA-treated animals, respectively, with blank values (no tissue) of about 300 cpm. Final values were expressed as picomoles of CO₂ per minute per nanogram of protein. In addition, a ratio of TH activity to DA content was used to estimate TH activity per DA terminal.

Protein. The protein content in the supernatant used for TH assay and in the synaptosomal fraction used for DA uptake was determined according to the method of Bradford (1976), whereas the protein content in tissue slices was estimated using the method of Lowry et al. (1951). Both assays were linear for up to 15 µg of protein.

Statistical analyses. All values are expressed as means ± SEM, and the number of repetitions of each experiment (n) is also indicated. Comparisons between 2 means were carried out by Student's *t* test (2-tailed). Comparisons between 2 patterns of DA release were made by a repeated-measures analysis of variance.

Results

DA efflux from adult striatum in control rats

Striatal slices were prepared from adult control rats and superfused with Krebs bicarbonate buffer. Considerable amounts of DA were released spontaneously from these slices during the initial 5 min of superfusion; however, this efflux declined during the subsequent 20 min to a low, stable rate of approximately 200 pg/min/mg protein (Fig. 1A). When these slices were then stimulated at 2 Hz, efflux increased approximately 3-fold during the first 20 min of continuous stimulation. DA overflow declined sharply during the next 10 min of stimulation, achieving a steady rate that was about 50–100% above the spontaneous

Figure 1. DA efflux from control striatal slices. DA content was measured in fractions collected every 5 min (*A*) without stimulation ($n = 3$) and (*B*) with 2 Hz stimulation (bar) beginning 25 min after the onset of superfusion ($n = 3$). Stimulation produced a significant increase in DA efflux ($F_{1,4} = 43.9$; $p < 0.01$).

rate (Fig. 1*B*). This pattern of stimulus-induced release was eliminated by removing calcium from the perfusion medium (see below).

DA efflux from neonatal striatum

DA efflux was examined using striatal slices from 7–10-d-old rat pups and from adult animals. DA overflow from neonatal slices developed more gradually than did overflow from adult slices. However, despite the fact that the DA concentration in the striatum of neonatal rats was only 20–40% that of adult control tissue, overflow from neonatal slices reached a value 2–3 times higher than that of the adult control rats within 20–30 min of stimulation (Fig. 2*A*). Moreover, when expressed as a fraction of the DA levels in freshly prepared slices, efflux from neonatal tissue was 5–6 times higher than efflux from adult tissue by the end of the stimulation period (Fig. 2*B*).

DA efflux from adult striatum in 6-HDA-treated rats

Adult rats given 6-HDA treatments had a 60–98% loss of striatal DA. An equivalent decrease in high-affinity DA uptake was observed in synaptosome-rich homogenates of striatum (Table 1), thus suggesting that the DA content per terminal did not change substantially and can serve as an index of the extent to which DA terminals have degenerated. In contrast, in a separate group of animals the loss of TH activity was significantly less

Figure 2. DA efflux from slices prepared from neonatal rats. DA efflux from striatal slices of 7–10-d-old rat pups ($n = 6$; shaded area) was compared with efflux from adult slices ($n = 10$; open area). Slices were superfused for 25 min under basal conditions and then subjected to 2 Hz stimulation for 30 min (bar). DA efflux is expressed (*A*) per milligram of protein and (*B*) as fractional efflux, i.e., the percentage of the DA content of adjacent slices prior to incubation. The DA content of slices from adult and neonatal rats was 82.3 ± 3.9 and 35.2 ± 4.5 ng/mg protein, respectively. Stimulated efflux was significantly greater than from neonatal slices, whether expressed per milligrams of protein ($F_{1,10} = 22.99$; $p < 0.01$) or as fractional efflux ($F_{1,10} = 197.96$; $p < 0.01$).

than the loss of DA, leading to an increase in the ratio of TH activity to DA content (Table 2; see also Zigmond et al., 1984).

When DA efflux was examined 3–4 weeks after the lesion, it was found that striatal slices from the brain-damaged rats responded to the onset of electrical stimulation by increasing the efflux. Considerably more DA efflux was observed than might have been predicted by the extent of DA loss from tissue, resulting in a significant increase in fractional DA efflux. The pattern of increase was different from that observed with control slices from adult rats, resembling, instead, that observed with slices from neonatal animals. Thus, during depolarization, DA efflux rose less rapidly at first than did efflux from adult control tissue, and exhibited little or no decline (Fig. 3). Greater increases in fractional DA efflux were observed in slices with larger lesions (Fig. 4).

DA efflux as a function of stimulus frequency

Because DA neurons normally tend to fire at a relatively low rate, with brief periods of increased activity (Grace and Bunney, 1979; Chiodo et al., 1980; Steinfels et al., 1983), we examined the response of striatal slices to sustained low-frequency stim-

Table 1. Striatal dopamine content and high-affinity uptake after 6-HDA lesions^a

Group (n)	DA content (ng/mg protein)	DA uptake ^b (pmol/mg protein/min)
Control (5)	87.4 ± 2.7	8.12 ± 0.53
6-HDA (5)	9.6 ± 2.8 (11.0%)	1.07 ± 0.24 (13.2%)

^a Animals received an intraventricular injection of 6-HDA (225 μ g) or vehicle solution after intraperitoneal pretreatment with desmethylimipramine (25 mg/kg, i.p.) and pargyline (40 mg/kg, i.p.) and were killed 3 weeks later. DA contents were measured in homogenates, and accumulations of 10 nM [³H]-DA were measured in crude synaptosomal fractions prepared from the same tissue sample. Numbers represent means $\pm$ SEM or percentages of sham-lesioned controls.

^b High-affinity DA uptake was calculated by subtracting uptake obtained in the presence of 0.1 mM unlabeled DA from the total tissue retention of tritium. In a single experiment, DA uptake was measured in the presence of 10 μ M nomifensine added 5 min prior to [³H]-DA and was found to be 0.80 pmol/mg protein/min.

Table 2. Dopamine and tyrosine hydroxylase activity in striatum after 6-HDA administration

Group (n)	DA content (ng/mg protein)	TH activity (pmol/mg protein/min)	TH/DA ($\times 10^3$)
Control (6)	76.0 ± 6.6	0.94 ± 0.08	11.9 ± 0.5
6-HDA (6)	4.1 ± 0.8 (5.4%)	0.11 ± 0.01 (11.7%)	28.2 ± 1.9 (234%)

Rats were killed 3 weeks after surgery. TH activity and DA content were measured in the same homogenate (see Materials and Methods). Values represent means $\pm$ SEM or percentages of sham-lesioned controls. TH activity and DA content were significantly lower in 6-HDA-treated animals than in controls ($p < 0.001$), whereas the ratio of these variables was significantly higher ($p < 0.001$).

Figure 3. DA efflux from slices prepared from 6-HDA-treated animals. DA efflux from striatal slices of 6-HDA-treated rats ($n = 3$; shaded area) was compared with efflux from vehicle-treated control animals ($n = 10$; open area). Striatal slices were superfused for 25 min under basal conditions and then subjected to 2 Hz stimulation for 30 min (bar). DA efflux is expressed (A) per milligram of protein and (B) as fractional DA release. Slices from 6-HDA-treated rats showed DA depletions of approximately 58% (mean $\pm$ SEM: vehicle, 82.3 ± 4.2 ng/mg protein; 6-HDA, 34.5 ± 2.4 ng/mg protein). Stimulated efflux was significantly greater than that from intact slices, as expressed per milligram of protein ($F_{1,6} = 22.42$; $p < 0.01$) and from 6-HDA-treated slices when expressed as fractional efflux ($F_{1,6} = 13.88$; $p < 0.01$).

ulation and to brief high-frequency stimulation. Slices prepared from adult control animals were exposed to 70 min of stimulation at 2 Hz, interrupted at 35 min by a 3 min period of stimulation at 20 Hz. Using this paradigm, we observed that once the initial 10–15 min period of rapid efflux was complete, slices maintained a relatively stable rate of calcium-dependent efflux in response to 2 Hz stimulation (Fig. 5). During the 5 min period initiated by the onset of 20 Hz stimulation, efflux increased 5-fold, while overflow increased almost 10-fold (Figs. 5A; 6).

Figure 4. Effect of 6-HDA-induced lesions on DA release from striatal slices. Rats received 6-HDA or vehicle and were killed 4 weeks later. Striatal slices were superfused for 25 min under basal conditions and then subjected to 2 Hz stimulation for 30 min (bar). Data are grouped by level of lesion-induced DA depletion, as determined from adjacent, nonincubated slices, and are expressed as fractional DA efflux as a function of time. In each group of 6-HDA-lesioned slices, fractional DA efflux was significantly greater than efflux from intact slices. Open circles, vehicle-injected controls ($n = 10$); closed circles, 50–80% DA depletion ($n = 3$) ($F_{1,6} = 13.88$; $p < 0.01$); open triangles, 80–96% DA depletion ($n = 6$) ($F_{1,10} = 85.91$; $p < 0.01$); closed triangles, 96% DA depletion ($n = 7$) ($F_{1,10} = 28.52$; $p < 0.01$).

Figure 5. DA efflux from control slices during low- and high-frequency stimulation. Striatal slices were superfused for 20 min without stimulation, then subjected to 2 Hz stimulation, interrupted after 35 min by a 3 min period of 20 Hz (bars). The superfusion medium consisted either of (A) normal Krebs buffer containing 1.25 mM CaCl_2 ($n = 9$) or (B) calcium-free buffer containing 5 mM EGTA ($n = 3$). Stimulation in the presence of calcium produced a significant increase in DA efflux ($p < 0.01$), with efflux at 20 Hz being significantly greater than at 2 Hz ($p < 0.01$). There was no significant effect of stimulation in the absence of calcium.

Figure 6. DA overflow from striatal slices during low- and high-frequency stimulation. Slices from adult intact (vehicle-treated) rats ($n = 9$), 6-HDA-treated rats (4 weeks postlesion; $n = 10$), and neonatal (7–10-d-old) rat pups ($n = 6$) were treated as described in the legend to Figure 5. DA overflow was calculated by subtracting the spontaneous efflux measured in the fraction collected 20–25 min after the beginning of superfusion from efflux collected during stimulation. DA overflow during 2 Hz stimulation delivered between 50 and 55 min (empty bars) was compared to the overflow produced by 3 min of 20 Hz stimulation, followed by 2 min of 2 Hz stimulation, delivered between 55 and 60 min (hatched bars). DA content in striatum of adjacent, noninjected control, 82.3 ± 3.9 ng/mg protein; of 6-HDA-lesioned, 5.1 ± 0.7 ng/mg protein; and of neonates, 35.2 ± 4.5 ng/mg protein.

Figure 7. Effect of stimulation on DA efflux and tissue DA content. At the end of the experiment described in the legend to Figure 6, the amount of DA recovered in the superfusate and the concentration of DA remaining in the tissue slices were determined. Shown are values for adult animals treated with 6-HDA or vehicle (*Veh.*) 4 weeks prior to the experiment, and for neonatal (7–10-d-old) rat pups. Fractional DA efflux from slices prepared from adult lesioned animals and neonatal pups was significantly greater than that from adult control animals ($p < 0.01$). DA depletion was significant only in slices from intact control animals ($p < 0.01$).

Fractional DA efflux from slices prepared from neonatal rats and stimulated at 2 Hz was higher than that observed from adult control slices, as has been shown previously. Efflux from neonatal slices increased significantly when slices were stimulated at 20 Hz, although the ratio of overflow to that at 2 Hz was only 2.8, less than in adult slices (8.5). Slices from 6-HDA-treated animals, on the other hand, showed a high fractional DA efflux in response to 2 Hz stimulation but no further increase in response to 20 Hz (Fig. 6).

After 60 min of stimulation, the total amount of DA lost to the superfusion fluid was calculated and the concentration of DA remaining in the tissue was determined. Slices from 6-HDA-treated rats and neonatal rats released an amount of DA equivalent to 80 and 50% of their total DA content, respectively, but no loss of tissue DA could be detected. By contrast, slices from control animals released the equivalent of only 16% of their DA content to the superfusion fluid, yet showed a 28% DA depletion (Fig. 7).

Increased release versus decreased reuptake

The increase in fractional DA efflux from striatum of 6-HDA-treated rats could be due to an increase in DA release by residual terminals, a decrease in DA reuptake from the synaptic cleft, or both. To determine the relative contributions of alterations in release and uptake, striatal slices were stimulated at 2 Hz for 15 min in the presence of normal buffer, and stimulation was continued in the presence of nomifensine (10 μ M), a treatment that inhibited DA uptake by 90% (Table 1). With slices from adult control rats, nomifensine produced an immediate increase in DA efflux, whereas there were much smaller responses with slices from the 6-HDA-treated rats (Fig. 8*A*). When these effects were expressed in terms of fractional efflux, DA efflux from control slices in the presence of nomifensine still was only about 50% of that observed from the other slices (Fig. 8*B*).

Discussion

Intraventricular injections of 6-HDA resulted in parallel decreases in the concentration of DA and the rate of high-affinity DA uptake in striatum, as has been noted previously (Uretsky and Iversen, 1970; Zigmond et al., 1984). 6-HDA treatment

Figure 8. Effect of nomifensine on DA efflux. Striatal slices from vehicle-injected control and 6-HDA-lesioned rats were superfused for 25 min under basal conditions and then exposed to 30 min of 2 Hz stimulation (*bar*). Nomifensine (10 μ M) was introduced into the buffer after the first 15 min of stimulation. *A*, DA efflux in a representative experiment as a function of time (standard medium, *hatched area*; nomifensine, *open area*). Arrow indicates introduction of nomifensine into the superfusion medium. *B*, Fractional rates of DA efflux from striatal slices during the 5 min immediately before (*open bars*) and after (*hatched bars*) addition of nomifensine (*nomi.*) from control ($n = 5$) and 6-HDA-lesioned ($n = 7$) rats. Nomifensine significantly increased efflux from slices from control animals ($p < 0.01$), but had no significant effect on efflux from slices from 6-HDA-treated rats ($p > 0.10$).

also decreased the rate of DA overflow from striatal slices, but this effect was considerably smaller than that of the loss of DA content, resulting in a significant increase in the fractional rate of DA overflow. The extent of this increase was related to lesion size. For example, a 60–80% depletion of striatal DA was associated with a 3.5-fold increase in fractional DA overflow, whereas 96% DA depletion was accompanied by a 7-fold increase.

The reduction in high-affinity DA uptake sites after 6-HDA treatment should contribute to the increase in fractional DA efflux. To assess this contribution, we measured DA efflux in control tissue after inhibiting high-affinity DA uptake with nomifensine and observed a 2-fold increase. However, fractional DA efflux in striatal tissue from brain-damaged rats was increased 3- to 7-fold. Thus, assuming that DA is normally inactivated by a nomifensine-sensitive process, it appears that there was an increase in the release of DA from residual terminals as well. These observations are reminiscent of our previous findings that, after 6-HDA-induced lesions in adult rats, there is in striatal tissue a disproportionately large concentration of dihydroxyphenylacetic acid (DOPAC), the deaminated metabolite of DA, and that the ratio of DOPAC to DA content increases exponentially as a function of DA depletion (Zigmond et al., 1984).

These observations suggest that partial destruction of DA terminals is accompanied by a significant increase in the capacity

of the residual terminals to deliver DA to distant sites. This increase may account for previous findings that lesions of the nigrostriatal bundle produce relatively few deficits in basal striatal function, and no gross behavioral dysfunctions, unless the loss of striatal DA is severe (Zigmond and Stricker, 1973; MacKenzie et al., 1985; Orr et al., 1986). Moreover, the present observation that striatal slices from 6-HDA-treated rats are unable to increase DA release in response to a large increase in stimulus frequency may be related to previous findings that rats with DA-depleting brain lesions, which behave normally in a neutral laboratory setting, show behavioral impairments when exposed to stressful stimuli, such as tail shock, that are known to release DA (Keller et al., 1983b; Snyder et al., 1985). Thus, these studies in isolated tissue may illuminate the behavioral syndrome that results from DA-depleting brain lesions.

The gradual development of the dopaminergic projection to striatum provides another condition under which the functional impact of DA hypoinnervation may be examined. Although the first neurites from substantia nigra reach the striatum early in development, innervation is not complete until about day 40 postpartum (Kirskey and Slotkin, 1979). In the present studies, striatal DA levels at days 7–10 were 20 to 40% of adult levels, but the rate of stimulated DA efflux was comparable to that seen in the adult, indicating a significant increase in fractional DA efflux. This enhanced capacity for DA release may account for previous findings that DA control over striatal neurons (Coyle and Campochiaro, 1976) and behavior (Lal and Sourkes, 1973; Baez et al., 1976) matures well before the adult density of dopaminergic innervation is attained.

The increases in DA overflow seen after lesions were induced in adult animals by 6-HDA, and in neonatal rats, apparently were accompanied by an increase in DA synthesis. This is indicated in each case by the failure of tissue DA levels to decline during stimulation and by the relative increase in TH activity (Porcher and Heller, 1972; Coyle and Campochiaro, 1976; Zigmond et al., 1984; and the present study). We conclude that the increased synthesis and release and the decreased reuptake of DA all serve to compensate for hypoinnervation after injury or during development.

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