**d-Amphetamine-Induced Depression of Central Dopamine Neurons:**
**Evidence for Mediation by Both Autoreceptors and a Striato-Nigral Feedback Pathway**

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**Summary.** The mechanism by which intravenous d-amphetamine (d-A) depresses the activity of dopamine (DA)-containing neurons in the substantia nigra was studied in anesthetized rats using single cell recording and microiontophoretic techniques. Kainic acid (KA) injections into the caudate nucleus were used to selectively destroy neuronal feedback pathways to the substantia nigra originating from the striatum. These lesions caused a five-fold increase in the amount of i.v. d-A needed to produce 50% inhibition of DA cells compared to control animals. Furthermore, in the lesioned animals even near lethal doses did not cause total abolition of the firing of DA cells. This was in marked contrast to unlesioned animals in which relatively low doses of d-A caused DA cells to temporarily cease firing entirely. Low non-convulsant doses of the GABA antagonist, picrotoxin, were found to reverse d-A-induced depression of DA cells in non-lesioned animals, but even high doses had no effect in lesioned ones. These results suggest that at low doses i.v. d-A produces its depressant effects on DA cells primarily through a striato-nigral feedback pathway, one link of which is GABAergic. At high doses d-A appears to inhibit DA cells through an action within the substantia nigra, perhaps at DA dendrodendritic synapses. Microiontophoretically applied dopamine was found to be equally potent in inhibiting DA cells in non-lesioned, lesioned, and picrotoxin-treated animals. Thus, the ability of microiontophoretically applied DA to inhibit DA cells appears to be mediated by an interaction with DA autoreceptors and not by the release of GABA from afferent terminals as has recently been suggested.

**Key words:** d-Amphetamine - Autoreceptor - Dopaminergic - Picrotoxin - Substantia nigra.

**Introduction**

In 1967 Corrodi and coworkers, based on biochemical evidence, suggested that d-amphetamine (d-A) might decrease the firing rate of dopaminergic (DA) neurons in the central nervous system. Various lines of evidence have shown that d-A increases release and blocks reuptake of DA at nerve terminals (Glowinski and Axelrod, 1965; Carlsson et al., 1966; Costa and Groppeiti, 1970; Von Voigtlander and Moore, 1973; Arnold et al., 1977). Corrodi et al. (1967) reasoned that such an action would increase the concentration of DA in the synaptic cleft thereby leading to an increased stimulation of postsynaptic DA receptors. They further suggested that some of the cells possessing these receptors might form part of a neuronal feedback pathway which may modulate DA cell activity. In 1973 we recorded from identified DA neurons in the zona compacta (ZC) of the substantia nigra which project to the striatum (Bunney et al., 1973a). In agreement with the feedback hypothesis, we found that intravenous d-A markedly depressed these cells at relatively low doses [D<sub>50</sub> (50% inhibition of firing rate) 1.60 ± 0.35 S.E.M. mg/kg]. Anatomical support for the feedback hypothesis is provided by several studies which have demonstrated the presence of at least two feedback pathways from the striatum to the substantia nigra (McGeer et al., 1974; Grofová, 1975; Hattori et al., 1975; Bunney and Aghajanian, 1976a). We have previously reported that selective lesions of these pathways greatly attenuate the inhibitory effects of i.v. d-A on ZC cell activity (Bunney and Aghajanian, 1973, 1976b). Anatomical (Hattori et al., 1973; McGeer et al., 1974), biochemical (Kim et al., 1971; McGeer et al., 1971; Hattori et al., 1973; Fonnum et al., 1974; Kataoka et al., 1974), and electrophysiological (Feltz, 1971; Precht and Yoshida, 1971; Crossman et al., 1973; Aghajanian and Bunney, 1974) evidence strongly suggests that at least one link in one of the feedback pathways uses gamma-aminobuty-
omic acid (GABA) as its neurotransmitter. We have also reported that in unlesioned animals picrotoxin (a putative GABA antagonist) reverses d-A inhibition of ZC neurons (Bunney and Aghajanian, 1976c).

Recently, another mechanism for d-A’s inhibitory effects on DA neurons has been proposed. It has been suggested that d-A releases dopamine from DA cell dendrites which make synaptic contact with other DA cells (dendrodendritic synapses) leading to self-inhibition of these neurons (Groves et al., 1975). Supporting this contention is anatomical (Hajdu et al., 1973; Bjorklund and Lindvall, 1975; Wilson et al., 1977) and electrophysiological (Wilson et al., 1977) evidence for dendrodendritic synapses within the substantia nigra; furthermore, there is evidence of dopamine release in the substantia nigra induced by potassium (Geffen et al., 1976), the indirect acting DA agonists —amphetamine and benztropine (Nieoullon et al., 1977a), electrical stimulation (Korf et al., 1976) and sensory stimuli (Nieoullon et al., 1977b). In addition, there is electrophysiological evidence for the presence of DA autoreceptors on DA cells in the ZC (Aghajanian and Bunney, 1976, 1977), and evidence of d-A inhibition of DA cell firing by d-A introduced locally into the substantia nigra by means of either a cannula (Groves et al., 1975, 1976) or microiontophoresis (Bunney and Aghajanian, 1977).

The purpose of the research reported here was to further examine the role that a striato-nigral feedback pathway may play in mediating d-A-induced inhibition of DA cell activity in the ZC of the substantia nigra. In addition, the possibility that GABA is one of the neurotransmitters used by neurons in such a pathway as well as the mechanism by which microiontophoretically applied dopamine inhibits ZC cells was investigated.

**Methods**

Two groups of male albino rats (Charles River Laboratories, Wilmington, MA) weighing 240—300 g were used. Both groups of animals were first anesthetized with chloral hydrate (400 mg/kg, i.p.). Then one group (N = 18) received a 1 µl unilateral injection of kainic acid (KA) (2.5 µg/µl in 0.9 % saline, pH 7.4) into the caudate nucleus (coordinate: A 8.9 mm, L 2.7 mm, V 5.4 mm, according to König and Klippel, 1970) by means of a stereotaxically placed Hamilton syringe with a 30 gauge needle (method of Coyle and Schwarcz, 1976). The other group of rats were administered in the same way a 1 µl unilateral injection of 0.9 % sodium chloride into the head of the caudate nucleus. Both injections were made over a period of 8 min. Great care was taken to remove any KA from the outside of the needle before introducing it into the brain. The needle was withdrawn slowly (0.5 mm every 5 min). These two precautions plus the long injection time were needed to prevent destruction of the cortex overlying the caudate nucleus due to KA traveling up the cannula tract.

Two to sixty-eight days following the injections the animals were again anesthetized with chloral hydrate and mounted in a stereotaxic apparatus. A 3 mm burr hole was then drilled in the skull at varying coordinates within the outer dimensions of the substantia nigra [lateral, 1200—2400 µm; anterior, 1270—2420 µm, according to König and Klippel, 1970]. In one series of experiments a single barrel micropipet with a 1 µm tip filled with 2 M sodium chloride (impedance at 1,000 hertz, 4—8 megohms) saturated with green dye (fast green) was then lowered through the burr hole. In another series of experiments, in which drugs were administered microiontophoretically as well as intravenously, a 5 barrel micropipet prepared as previously described (Haigler and Aghajanian, 1974), and broken back to a tip diameter of 4—5 µm was used (Aghajanian and Bunney, 1977). Each barrel was filled by direct injection using the method of Asaki et al. (1968). The center (recording barrel) was filled with 2 M NaCl saturated with fast green. One side barrel, used to automatically balance tip current (Salmoiraghi and Weight, 1967), was filled with 4 M NaCl. Dopamine hydrochloride (0.1 M, pH 4.0) and GABA (0.01 M in 0.1 M NaCl, pH 4.0) were carried in two of the side barrels. In vitro resistances measured at 60 Hz were 90—100 megohms in each of the drug barrels, 10—15 megohms in the balance barrel and 4—8 megohms in the recording barrel. A retaining current of 10 nA was used for all neurotransmitters and drugs.

Electrode potentials were passed through a high input impedance amplifier and monitored on an oscilloscope. Integrated firing was recorded by means of a rate averaging computer (Aghajanian et al., 1970). Body temperature was continuously monitored and maintained at 36—38°C. Drugs were applied microiontophoretically or administered i.v. after recording a baseline firing rate for at least 5 min. When drugs were given i.v. only one cell was recorded from to avoid possible residual drug effects. All drug dosages are given in terms of the weight of their salt. Recording sites were marked by passing a 30 nA negative current through the recording barrel for 10 min resulting in the deposition of a spot of fast green (50 µ dia.) (Thomas and Wilson, 1965). The rats were then perfused with a solution of 10 % buffered formalin phosphate. Serial frozen sections were cut at 50 µ intervals and stained with neutral red. In addition to verifying the location of the recording tip by locating the green spot, the extent of damage secondary to the KA injection was determined in each animal injected with this amino acid.

In three KA injected animals, after recording was completed, the brains were rapidly removed and chilled to 0°C. The substantia nigra was then removed by a punch technique (Palkovits, 1973) from approximately 1 mm thick nigral slices (A1610—2580 µ; König and Klippel, 1970) and frozen at —70°C until assayed. GABA was assayed by binding to synaptic membranes treated with 0.05 % Triton X-100 (modified method of Enna and Snyder, 1976).

**Results**

**KA Lesions**

KA injections resulted, as previously described (Coyle and Schwarcz, 1976), in spontaneous contralateral turning for approximately 48 h followed by ipsilateral turning for another 48 h. During the first 24 h after injection, animals lost up to 15 % of their body weight due to aphasia and adipsia. If left to feed themselves over 50 % died. Once this fact was ascertained all subsequent KA lesioned rats were fed a 1 % glucose solution by means of a baby bottle for the first 48 h. After this time they resumed eating and drinking spontaneously and rapidly caught up in weight to their unlesioned litter mates. Mortality rate in these animals was 12 %.