In Vivo Voltammetric Study of Dopamine Release in the Striatum Following Microinjection of Apomorphine into the Substantia Nigra Zona Reticulata

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Abstract—Effect of microinjection of apomorphine into the substantia nigra zona reticulata (SNR) on the dopamine (DA) release in the ipsilateral striatum was examined in the present study. To measure the DA release, we determined DA and/or DOPAC levels in the striatum by using in vivo voltammetry. The microinjection of apomorphine (10 μg/2 μl) into the SNR produced a rapid and prolonged increase in the DA and/or DOPAC peak in the ipsilateral striatum. These results suggest that release of DA from dendrites of DA cells normally plays a physiological role in the SNR neurons.

It has been reported that a low dose of apomorphine inhibits not only DA cell firing but also the SNR non-DA cell firing rate (1–7). The inhibitory action of apomorphine on SNR neurons is blocked by striatoniigral pathway transection (3), indicating that inhibitory action of apomorphine on SNR neurons may be involved in the striatoniigral system. The SNR receives input from the neostriatum and relays this information either directly or via interneurons to the substantia nigra zona compacta (SNC) (8–11). In addition, dendritic arborization from DA neurons in the SNC form synaptic contacts with SNR cells (12, 13). These and other lines of evidence (14–17) suggest a close functional relation between SNC and SNR neurons. For example, unilateral intranigral GABA or GABA agonist produces contralateral circling behavior (18–23) which is reduced by pretreatment with haloperidol (18, 20). In fact, GABA applied iontophoretically into the SNR increases the spontaneous firing of the SNC DA cells probably due to disinhibition (14), and intranigral muscimol, a GABA agonist, stimulates DA release in the caudate nucleus (19). On the other hand, Waszczak and Walters have reported iontophoretically applied DA markedly attenuates the inhibition of SNR neurons by applied GABA (24). Recently, they extended their findings by providing evidence that the modulatory effect of DA is significantly enhanced in rats which received 6-hydroxodopamine (6-OHDA) lesions of the nigrostriatal DA neurons (25). The purpose of the present study was to characterize the response of SNR neurons to microinjection of apomorphine, a DA agonist. If SNR neurons are inhibited by microinjection of apomorphine, then disinhibition of SNC DA cells might have occurred. To measure the activity of nigrostriatal dopaminergic neurons, we employed the in vivo voltammetric technique which is able to detect the release of DA in the striatum.

All experiments were carried out on male Wistar rats weighing 250–300 g. The method for surgery (26) and recording electrochemical signals (27, 28) used were described previously. Briefly, a three electrode system was used for differential pulse voltammetry (27–31). A carbon fiber was encased in a
glass microcapillary filled with carbon paste and was used as a working electrode. The electrochemical surface of the working electrode is a carbon cylinder of 5 µm radius that protrudes from the glass capillary by 500 µm. Electrochemical modification was accomplished in phosphate-buffered saline (pH 7.4) solution by application of a triangular wave (70 Hz, 0.0 to 3.0 V vs. an Ag/AgCl electrode) for 20 sec, followed by another 10 sec period during which the potential was held at 1.5 V. Electrochemically-modified cylindrical electrodes were then preconditioned and tested prior to in vivo use with differential pulse voltammetry in 200 µM ascorbic acid and 20 µM 3,4-dihydroxyphenylacetic acid (DOPAC) until a stable response was obtained. The animals were anesthetized with chloral hydrate (400 mg/kg, i.p.), placed on a stereotaxic apparatus and subsequently subjected to the surgical operation. The body temperature was maintained at 37±0.5°C. Following surgery to expose the skull, holes were drilled over the caudate nucleus (approximately 2.0 mm anterior and 2.5 mm lateral to bregma) and the substantia nigra (2.8-3.0 mm anterior from lambda and 2.0 mm to the midline). The locus for the electrode insertion was determined according to the stereotaxic atlas of König and Klippel (32). The working electrode was inserted into the caudate nucleus, and the reference electrode and auxiliary electrode were placed on the dura surface of the frontal cortical area. Recording parameters of differential pulse voltammetry were as follows: potential range, -0.1 to +0.45 V; pulse amplitude, 50 mV; scan rate, 25 mV/sec. At 1.5 hr after the implantation of the electrode, measurements of electrochemical signals were started, and after another 30 min, microinjection of apomorphine into the SNR was initiated. Apomorphine was microinjected at a rate of 2 µl/5 min. At the end of the experiments, the animals were sacrificed under deep pentobarbital-Na anesthesia and perfused with 10% formalin. Frozen 50 µm thick sections of the whole brain were cut using a freezing microtome (MA-101, Komatsu). The location of each site was verified. Only the rats in which the infusion target was confirmed histologically were included in the analysis of the electrochemical data. Student's t-test was used for statistical evaluation of the data. The 0.05 level of probability was accepted as significant.

Figure 1 shows a typical series of voltamograms recorded in the caudate nucleus showing clear separation of DA and/or DOPAC (peak 1) and 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and/or uric acid (peak 2) signals. Each peak was seen at +0.1 V and +0.3 V, respectively. Since ascorbic acid peak sometimes overlapped with the DA and/or DOPAC peak, current (+0.05) was applied through the working electrode for 5 min just before measurements. A microinjection of apomorphine (10 µg/2 µl) into the SNR produced a marked increase in DA and/or DOPAC peak, but not the 5-HT, 5-HIAA and/or uric acid peak in the ipsilateral caudate nucleus. Mean percent increases from the baseline in the present experiments are summarized in Fig. 2. As can be seen in Fig. 1 and Fig. 2, respectively, a low dose of apomorphine (10 µg/2 µl) produced a rapid and sustained increase in the DA and/or DOPAC peak, but not the 5-HT, 5-HIAA and/or uric acid peak in the ipsilateral caudate nucleus; the effect began about 20 min after microinjection. The maximum increase in the DA and/or DOPAC peak was 80 min after microinjection, and then it declined slowly toward baseline during the next hour.

The present experiments have demonstrated that microinjection of apomorphine into the SNR produced a marked increase in the DA and/or DOPAC peak in the ipsilateral caudate nucleus. Recently, Ikeda et al. have reported that when the oxidation current of ascorbic acid is large enough to influence that of DOPAC, the peak for ascorbic acid can be removed by holding the voltage (0.05 V)
Changes in the electrochemical signals recorded from the caudate nucleus following microinjection of apomorphine (10 μg/2 μl) into the ipsilateral SNR. At the arrow, apomorphine was infused. The vertical axis shows differential pulse oxidation current.

between the working and the reference electrodes at a higher oxidation potential than that for ascorbic acid before each measurement, to allow clear monitoring of the DOPAC signal, being lower than the oxidation potential of DOPAC (33). The electrochemical signals that we obtained with differential pulse voltammetry clearly indicate that ascorbic acid or 5-HT, 5-HIAA and/or uric acid is not contributing to the signal at the DA and/or DOPAC peak. Since the ascorbic acid peak could be removed by applying current just before the measurements and the 5-HT, 5-HIAA and/or uric acid peak was not influenced by microinjection of apomorphine into the SNR, only the DA and/or DOPAC peak was influenced by microinjection of apomorphine, indicating that microinjection of apomorphine into the SNR can produce an increased release of DA in the ipsilateral striatum. Furthermore, in the preliminary experiments, the DA and/or DOPAC peak was increased by treatment with haloperidol and 5-HT, 5-HIAA and/or uric acid peak was markedly increased by 5-hydroxytryptophan, indicating that changes in DA and/or DOPAC peak height reflect nigro-striatal dopaminergic activity.

A growing body of evidence indicates that the SNR neurons modulate the activity of SNC neurons via inhibitory processes. Direct infusions of various compounds into the SNR, for example, have been reported to alter rotational behavior in rats with unilateral...
lesions of the DA nigrostriatal pathway (18–23). In addition, several studies suggest that SNR and SNC neurons display reciprocal firing patterns both spontaneously and in response to certain drugs (14, 17). SNR neurons receive a GABAergic input from the neostriatum (9, 10, 34) and are very sensitive to inhibition by GABA (3, 14, 17). Recently, Waszczak and Walters have reported iontophoretically applied DA markedly attenuates the inhibitions of SNR neurons by applied GABA (24). Furthermore, they extend their findings by providing evidence that the modulatory effect of DA is significantly enhanced in rats which receive 6-OHDA lesions of nigrostriatal DA neurons (25). Therefore, one may predict that release of DA from dendrites of DA cells normally plays a physiological role in modifying SNR response to GABA. If all SNR neurons control the activity of SNC DA neurons via inhibitory processes, the following sequence of events is predicted by the microinjection of apomorphine into the SNR: Microinjection of apomorphine into the SNR interrupts the neurotransmission of the striatonigral GABAergic pathway by attenuating SNR response to GABA; the decreased response of SNR neurons to GABA produces hyperactivity of SNR neurons; the increased activity of SNR neurons may send much more inhibitory impulses to SNC DA neurons; the inhibitory impulses may cause a hypoactivity of the nigrostriatal DA pathway. However, an unexpected increase in the DOPAC peak in the striatum by microinjection of apomorphine into the ipsilateral SNR was found in the present experiments. Our results lend additional support to the notion of regional differences among SNR neurons. Furthermore, the DA and/or DOPAC peak was not decreased by the microinjection of apomorphine, indicating that DA autoreceptors on the dendrites of DA cells in the SNC were not affected by the microinjected drug.

Recently, we have reported that SNR...
neurons display two types of patterns in response to amphetamine (35). Whereas the large majority of SNR neurons are excited by the challenge injection of amphetamine, the remaining SNR neurons are inhibited by the drug. It is conceivable, therefore, that all SNR neurons are not homogenous in their response to amphetamine or apomorphine. It is likely, therefore, that apomorphine might act on some SNR interneurons and cause disinhibition of DA cells. The disinhibition of DA cells following microinjection of apomorphine into the SNR might be involved in the increased DOPAC peak in the striatum. Further understanding of the mechanisms underlying these changes in the DOPAC peak in the striatum may shed new light on the neuronal systems and processes mediating the behavioral alterations produced by micro-injection of apomorphine into the SNR.

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