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# Prolactin-Induced Yawning Behavior Requires an Intact Nigro-Striatal Dopamine System

N. J. LAPING AND V. D. RAMIREZ[1]

*Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801*

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LAPING, N. J. AND V. D. RAMIREZ. *Prolactin-induced yawning behavior requires an intact nigro-striatal dopamine system.* PHARMACOL BIOCHEM BEHAVIOR 29(1) 59–62, 1988.—Herein, we evaluate the importance of the nigro-striatal dopamine system in prolactin-, apomorphine-, and physostigmine-induced yawning behavior. Bilateral 6-OH-dopamine lesions of the substantia nigra were performed on male rats (2–4 months old). The lesioned as well as control rats were injected with either physiological saline, physostigmine (200 μg/kg), apomorphine (50 μg/kg), or ovine prolactin (0.25 μg/kg) 72 hours after the surgical procedure. The results show that bilateral lesions of the substantia nigra did not affect physostigmine-induced yawning whereas both apomorphine- and prolactin-induced yawning were reduced by the lesion. Following the observation period the caudate nuclei were removed and analyzed for dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) content. The lesions reduced DA and DOPAC content in all treatment groups compared to the respective intact groups. Also, both DA and DOPAC concentrations were lower in the intact apomorphine and prolactin treated groups compared to intact saline controls, at times that were temporally related to the display of yawning behavior suggesting a decrease in dopamine activity following apomorphine and prolactin treatment. Interestingly, DA and DOPAC concentrations were higher in the lesioned apomorphine group compared to lesioned saline controls; however, in the lesioned prolactin group only the DA concentrations were higher when compared to lesioned saline controls. These results indicate that prolactin- and apomorphine-induced yawning require an intact nigro-striatal dopamine system and that these substances induce yawning by different mechanisms.

Apomorphine Caudate nucleus Dopamine Prolactin Substantia nigra Yawning 6-OH-Dopamine lesions

YAWNING behavior, a discrete event with a low spontaneous occurrence, appears to be a well suited model to study behaviors linked to dopaminergic systems such as the nigro-striatal dopamine system. Yawning is also of clinical interest since it has been reported that psychotics rarely yawn [20], and yawning is symptomatic in a wide range of central nervous system disorders such as brain lesions, chorea, and encephalitis [2, 14, 17]. It has been shown that a variety of substances, such as dopamine (DA) agonists, cholinergic agonists, as well as peptide hormones, induce yawning behavior [9, 10, 22, 24, 27, 29–31]. A prevalent view of the regulation of yawning considers that cholinergic neurons which stimulate yawning are under inhibitory control by dopaminergic neurons. Yawning evoked by cholinomimetics, such as physostigmine or pilocarpine, are thought to be mediated by central muscarinic receptors, since yawning induced in this manner is blocked by scopolamine hydrobromide [29]. Furthermore, it is known that the striatal cholinergic system can be inhibited by dopamine [1, 25, 28]. In light of this, it is not surprising that agents which inhibit central dopamine systems induce yawning behavior as well. For example, low doses of dopamine agonists, such as apomorphine (APO) or piribedil (which are thought to be preferentially stimulate dopamine autoreceptors at low doses, and thereby inhibit the dopamine system) can induce yawning behavior as well as the stretch-yawning-syndrome [12, 22, 24, 27, 31]. Interestingly, scopolamine also blocks yawning, when induced with APO or piribedil [16,31]. In terms of dopaminergic agents, yawning behavior is thought to be evoked by compounds which activate dopamine autoreceptors, as illustrated when autoreceptor-selective drugs are used such as 3-PPP or TL-99 [15,23]. However, yawning behavior is also induced by protein hormones from the pituitary, such as ACTH, oxytocin, and alpha MSH [9, 10, 21]. We have shown that low doses of ovine prolactin (PRL) [0.25 μg/kg] injected systemically induce yawning in young adult male rats [19]. In spite of the initial release of dopamine by PRL from the striatal terminals [6], we have hypothesized that the initial release of dopamine eventually will bind to dopamine autoreceptors and then induce yawning behavior. Since there was an indication that infusions of prolactin into the caudate nucleus can induce yawning as well [6], and that 6-OH-Dopamine (6-OH-DA) lesions of the striatum abolished

[1]Requests for reprints should be addressed to V. D. Ramirez, Department of Physiology and Biophysics, University of Illinois, 524 Burrill Hall, 407 S. Goodwin, Urbana, IL 61801.

APO-induced yawning in rats [7], it was of interest to investigate if the nigro-striatal dopamine system is involved in prolactin- as well as APO-induced yawning behavior. Herein, we report the effect of bilateral 6-OH-DA lesions of the substantia nigra on physostigmine-, apomorphine-, and prolactin-induced yawns.

## METHOD

Male Holtzman albino rats, 270–450 g, served as subjects. The animals were kept on a constant 14:10 hr light-dark cycle, (lights off at 19:00), in clear plastic cages with Sanicell as bedding, and were fed with rat chow pellets and water ad lib.

Six-OH-DA lesions were performed by acutely implanting a 30 g needle into the rostral and dorsal area of each substantia nigra: 2.9 mm posterior from the bregma; 2.2 mm lateral from the superior sagital sinus; and 7.5 mm ventral from the dura mater (according to DeGroots rat atlas). Six-OH-DA hydrochloride (Sigma) was dissolved in phosphate buffered saline (pH 7.6) to a final concentration of 20 $\mu$g base/$\mu$l. One $\mu$l was infused over a period of two to three minutes using a slow peristaltic pump (Rabbit peristaltic pump by Rainin instruments Co.). Lesions were performed on animals under ketamine anesthesia (100 mg/kg). Three days after this lesion hypermotility and stereotyped behavior was observed in the majority of animals.

Seventy two hours after the lesions were made the intact as well as lesioned animals were tested with either 100 $\mu$l physiological saline, physostigmine (Sigma) 200 $\mu$g/kg, apomorphine hydrochloride (Sigma) 50 $\mu$g/kg, or ovine prolactin (Sigma, lypholized and dissolved in 0.1 M $NaHCO_3$) 0.25 $\mu$g/kg. All compounds were injected subcutaneously between 14:00–16:00 hr, using 50 $\mu$l/100 g body weight.

Animals were placed in clear plastic cages (45×26×20 cm) with Sanicell as bedding, and observed for the occurrence of yawns as well as stretch-yawns for 60 minutes, (except for the apomorphine group, which was observed for only 30 minutes, due to its greater potency). For simplicity, yawns will encompass stretch-yawns as well as yawns.

Sixty minutes after the drug injection the subjects were given ether anesthesia and were decapitated. An additional APO group (APO-30′) was killed 15–30 minutes after the APO injection in order to measure DA and DOPAC content when yawning activity is greatest for this drug. It was anticipated that APO would have its greatest effect on the dopaminergic system at this time. Caudate nuclei (CN) were dissected out and individually weighed, placed in 0.1 N perchloric acid with 0.4 mM $NaHSO_3$, and homogenized with an ultrasonic sonicator. The samples were then centrifuged at 1700 g for 15 minutes and the supernatant was injected into a High Pressure Liquid Chromatography system linked to an electrochemical detector to measure DA and DOPAC content which is expressed as ng/mg tissue [4,5]. Standards for DA and DOPAC were prepared from dopamine hydrochloride (Sigma) and 3,4-dihydroxyphenylacetic acid free base (Sigma). The column used in separating the catechols was a biophase ODS column, using 5 $\mu$m spheres from BioAnalytical Systems Inc. Values from the left and right CN were averaged for each animal.

Significant differences between groups (alpha<0.05) were determined by Kruskal-Wallis one way ANOVA, followed by Mann-Whitney U-tests. Values are expressed as mean ± standard error.

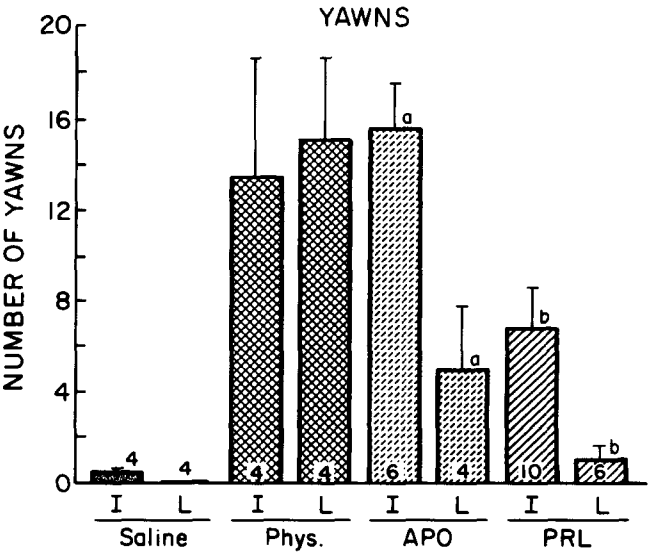


FIG. 1. Number of yawns per 60 minutes except for the apomorphine group which is per 30 minutes. Physostigmine induced yawning is not affected by a 6-OH-DA lesion. Both apomorphine and prolactin induced yawning are significantly decreased by a lesion of the nigro-striatal dopamine system. Mean ± standard error are shown. Numbers within bars show the number of subjects in each group. I—Intact; L—Lesion. Same lower case letters indicate significant differences within the same treatment ($p<0.05$, Mann-Whitney U-test).

## RESULTS

In intact saline treated animals yawning behavior occurred at a low spontaneous frequency of 0.5±0.3 yawns/hr, ranging between 0 and 2 yawns/hr. Lesioned animals treated with saline showed no yawns during this observation time. Physostigmine, a cholinergic agonist, induced 13.5±5.0 yawns/hr. This phenomenon was not abolished by a bilateral lesion of the substantia nigra (15.0±4.5 yawns/hr). In contrast, both APO- and PRL-treated animals showed significantly fewer yawns when lesioned ($p<0.05$). The APO group dropped from 15.5±2.8 to 5.0±3.5 yawns/30 min and the PRL group from 6.9±2.3 to 1.0±0.6 yawns/60 minutes (see Fig. 1).

To determine the success or quality of the lesion, the caudate nuclei were removed from the lesioned as well as from the intact animals after the observation period and were analyzed for DA and DOPAC concentrations (see Table 1). Interestingly, the intact physostigmine, intact APO-30′, and intact PRL group showed significantly lower DA concentrations as compared to those of the intact saline group ($p<0.05$). But the DA and DOPAC levels of the APO-treated animals killed 60 minutes after the APO injection (APO-60′ group) were not significantly lower. Clearly, a lesion of the substantia nigra caused a significant reduction in DA concentrations of the caudate nucleus in all treated animals as compared to those of intact animals (see Table 1). Within the lesion groups, only physostigmine treatment reduced the DA content values significantly compared to lesioned saline controls (0.5±0.1 vs. 1.4±0.3). Curiously, the lesioned APO-60′ group and the lesioned PRL group had significantly higher DA concentrations than those of the lesioned saline group (5.6±1.0 and 4.4±0.3 vs. 1.4±0.3, respectively).

Only the DOPAC concentration values for intact APO-30′ and PRL groups were significantly lower from those of the

TABLE 1

DOPAMINE (DA) AND DIHYDROXYPHENYLACETIC ACID (DOPAC) CONCENTRATIONS IN THE CAUDATE NUCLEUS OF YOUNG ADULT MALE RATS FROM INTACT AND BILATERAL SUBSTANTIA NIGRA-LESIONED ANIMALS (6-OH-DA)

| Condition | n | Treatment | DA (ng/mg wet weight) | DOPAC |
|---|---|---|---|---|
| Intact | 4 | Saline | 17.6 ± 0.9 | 4.6 ± 0.3 |
| | 4 | Physostigmine | 13.0 ± 1.0* | 4.7 ± 0.3 |
| | 6 | APO-30′ | 9.4 ± 0.6* | 2.4 ± 0.2* |
| | 6 | APO-60′ | 15.4 ± 1.0 | 4.0 ± 0.2 |
| | 10 | Prolactin | 11.5 ± 0.6* | 3.8 ± 0.2* |
| Lesion | 4 | Saline | 1.4 ± 0.3 | 0.5 ± 0.1 |
| | 4 | Physostigmine | 0.5 ± 0.1* | 0.3 ± 0.0 |
| | 5 | APO-30′ | 0.6 ± 0.2 | 0.2 ± 0.1 |
| | 4 | APO-60′ | 5.6 ± 1.0* | 2.3 ± 0.3* |
| | 6 | Prolactin | 4.4 ± 1.3* | 0.8 ± 0.2 |

Asterisks indicate significant difference from respective saline group (Mann-Whitney U-test, $p<0.05$). All lesioned groups are significantly lower in DA and DOPAC concentrations when compared to respective intact groups ($p<0.05$). Notice that in the lesioned animals, APO-60′ and PRL groups had significantly higher DA levels than the lesioned saline controls.

intact saline group (see Table 1). As with the DA concentration values, all lesioned groups showed significantly lower DOPAC concentrations when compared to their respective intact groups ($p<0.05$). Within the lesioned groups the APO-60′ group had significantly higher values of DOPAC compared to those of the saline group (2.3±0.3 vs. 0.5±0.1 ng/mg).

## DISCUSSION

Our results indicate that physostigmine-induced yawning is not reduced by a bilateral lesion of the substantia nigra, whereas both APO- and PRL-induced yawning are. Physostigmine most likely mimics cholinergic system involvement in yawning stimulation. However, the present results show that physostigmine does reduce DA concentrations in the caudate nucleus in intact as well as lesioned rats. This reduction may be due to cholinergic activation of GABA, which can inhibit dopamine neurons [13]. In any event, different sites of action appear to be responsible for physostigmine-induced yawning versus APO- and PRL-induced yawning.

Also in the intact animals, both DA and DOPAC concentrations in the caudate nucleus were lower in the APO-30′ and PRL groups as compared to those of saline controls, at times that were temporally related to the yawning display. It has been proposed that one site in the central nervous system where APO and PRL act to induce yawning behavior is the nigro-striatal dopamine system. In support of this hypothesis is evidence indicating that not only do infusions of APO within the caudate nucleus decrease striatal neuron firing rates [3], but that a local infusion of APO is also capable of inducing yawning [8]. The concentrations of APO used in the present experiment could act on the nigro-striatal dopamine autoreceptors, given that APO at these low doses binds preferentially to dopamine autoreceptors. Activation of these autoreceptors could inhibit synthesis and/or decrease release of dopamine [11] thereby lifting dopaminergic inhibition of cholinergic systems which have been demonstrated to activate yawning [30].

With regard to PRL, intra-striatal but not intra-accumbens infusions of PRL can induce yawning ([6], unpublished observations). It is proposed that PRL induces yawning by a similar mechanism to that of APO. However, this mechanism is probably secondary to its initial releasing action of DA as indicated by the longer latency of PRL-induced yawning [19]. In this way small doses of PRL can release small amounts of DA from the caudate nucleus [6], and ultimately decrease dopaminergic activity through autoreceptors after a certain latency period. In support of the temporal differences between APO- and PRL-induced yawning is the finding that DA and DOPAC concentrations had recovered by 60 minutes in the intact APO group but not in the intact PRL group.

It can be speculated that in the lesioned animals the remaining intact neurons must increase their output to compensate for the loss of functional dopamine neurons. This could be accomplished in part by a decrease in dopamine auto-receptors which leads to a decrease in feedback inhibition and/or by an increase in receptors at the postsynaptic site leading to hypersensitivity [18]. The results of the APO-30′ groups suggest that the number of functional autoreceptors have decreased in the lesioned condition because in this group the DA and DOPAC concentrations were not significantly lower than the lesioned saline controls. In the lesioned APO-60′ group the DA and DOPAC concentrations were much higher than the lesioned saline controls. A lesion of the nigro-striatal dopamine system can induce postsynaptic hypersensitivity [18], which was observed in these experiments by the display of stereotyped behavior in the majority of lesioned animals. However, such hyperactivity could also be the result of DA release from degenerating nerve terminals as well as decreased DA reuptake. In the lesioned condition low doses of APO may in fact stimulate postsynaptic receptors. The activation of postsynaptic receptors could inhibit the GABA-ergic inhibitory feedback loop [13], resulting in an increased impulse flow of the nigro-striatal dopamine neurons. It has been shown that the increased impulse flow of dopaminergic neurons can result in an increase in DA synthesis [26]. Thus as it was the case one could expect higher DA and DOPAC concentrations 60 minutes after the APO injection.

PRL releases DA and with the decreased number of functional autoreceptors less effective feedback inhibition would be observed. Again, the transient release of dopamine caused by PRL could have inhibited the GABA-ergic feedback loops, resulting in increased impulse flow of dopamine neurons. This could contribute to the greater DA concentrations observed in the lesioned PRL group compared to the lesioned saline group. However, in this group no increase in DOPAC concentration was observed.

In summary, physostigmine-induced yawning does not require an intact nigro-striatal dopamine system. In contrast, APO- and PRL-induced yawning behavior requires an intact nigro-striatal dopamine system. But, the mechanism of action of these two substances appear to be different. The findings that PRL in addition to other pituitary hormones such as ACTH, MSH, and oxytocin, can induce yawning behavior [9, 10, 21], suggests that the nigro-striatal dopamine system may be an important site where pituitary hormones modulate behaviors.

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