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Prostaglandin Inhibition of Apomorphine-Induced Circling in Mice

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SCHWARZ, R. D., N. J. URETSKY AND J. R. BIANCHINE. *Prostaglandin inhibition of apomorphine-induced circling in mice.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1233–1237, 1982.—The effect of prostaglandins (PGs) on apomorphine (apo)-induced circling was examined in unilaterally lesioned mice. Intraventricularly injected PGD_2, PGE_2, and $PGF_{2\alpha}$ at a dose of 1.0 nmole/g all inhibited apo-induced circling. When injected directly into the striatum, these same PGs also inhibited circling in a dose range of 0.01–0.1 nmole/g, while the PGE_2 metabolite, 13,14-dihydro-15-keto-PGE_2, was inactive at 0.1 nmole/g. For both routes of administration, $PGF_{2\alpha}$ appeared to be the most potent of the PGs tested. PGs administered alone by either route to unilaterally lesioned mice did not produce circling. Pretreatment with the PG synthetase inhibitor, indomethacin, caused the apo treated mice to circle at significantly higher rates than control animals. These results are the first report suggesting that within dopamine (DA)-mediated pathways PGs act at sites postsynaptic to the dopaminergic synapse.

Prostaglandins Apomorphine Circling behavior Indomethacin 6-Hydroxydopamine

ALTHOUGH prostaglandins (PGs) have been shown to be present in the CNS and to alter CNS activity [22], their exact role within neuronal pathways controlling motor function is currently unclear. Previous studies indicate that PGs might affect dopamine (DA) neurotransmission which has been shown to be intimately involved in motor function. Central PG administration has been shown to produce catelepsy [7] and block conditioned avoidance responding [10], effects which are similar to those produced by DA receptor blockers. In addition, the central administration of PGs has been shown to produce a decrease in food intake [13], an effect that may have features in common with the decrease in food intake following lesioning of the DA nigrostriatal pathway [17]. The finding that PGs are found in the caudate nucleus, the area of the brain richest in DA synaptic connections [1], suggests that endogenous PGs may play a physiological role in the modulation of motor function regulated by dopaminergic neurotransmission.

Recently we reported that centrally injected PGs had the ability to inhibit amphetamine (amph)-induced circling in mice. PGD_2, PGE_2, and $PGF_{2\alpha}$ inhibited circling when administered either intraventricularly or intrastriatally [14,15]. Circling produced by amph has been shown to be the result of dopamine release from intact nerve terminals in unilaterally lesioned animals [11]. Thus, PGs might inhibit amph-induced circling by inhibiting DA release. This idea is consistant with experimental results obtained in the peripheral nervous system showing that PGs of the E series inhibit norepinephrine (NE) release [2,6].

However, another explanation for the PG inhibition of circling produced by amph, is that the PGs act at sites postsynaptic to the DA synapse. It is known that direct acting DA agents, such as apomorphine (apo), cause circling in unilaterally lesioned animals by direct stimulation of the DA receptor [18,19]. The ability of agents to inhibit apo-induced circling in mice would therefore suggest a postsynaptic site of action. The following results are the first report giving direct evidence for the idea that within the DA neuronal pathway, PGs can act at sites postsynaptic to the DA synapse.

METHOD

6-Hydroxydopamine Lesion in Mice

Swiss-Webster, male mice (20–30 g) were lesioned in the left striatum with 6-hydroxydopamine HBr (6-OHDA) while under chloral hydrate anesthesia (400 mg/kg, IP). Sixteen μg of 6-OHDA HBr were dissolved in 4 μl of ice-chilled 0.9% saline containing 1.6 μg of ascorbic acid and were injected into the brain over a 4 min period. Coordinates for the injection site were: 5 mm anterior to the occipital suture (lambda), 2.1 mm lateral to the midline and 3.5 mm below the surface of the skull. Five to 7 days after surgery, all mice were injected with D-amph (4 mg/kg, IP). Only those mice that circled toward the side of the lesion at a rate of 10 turns/min were retained for further testing which was then performed 14 days after surgery. Circling was measured visually by placing the mice in a 2 liter round bottom flask and recording both the direction of turning and number of 360° turns within consecutive 5 min time periods.

Electrolytic Lesion in Mice

In another group of mice, an electrolytic lesion was made in the left striatum at the same coordinates as the 6-OHDA lesion (see above) with a current of 2.5 mA applied through a stainless steel insect pin (insulated except at the tip) for a period of 20 sec. The animals were allowed 3–4 days to re-

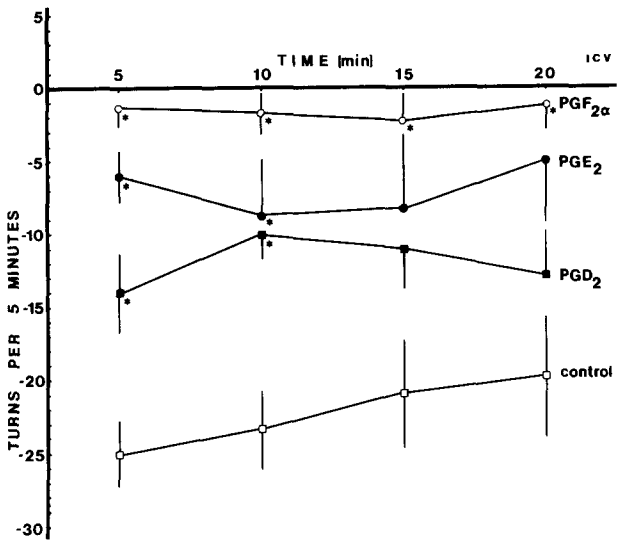

FIG. 1. Effect of intraventricularly injected prostaglandins on apomorphine-induced circling. Mice, previously lesioned with 6-OHDA in the left striatum, were administered saline or PG (1.0 nmole/g) intraventricularly followed 10 min later by apo (1.0 mg/kg, IP). Turns were counted for 5 min periods beginning 5 min after apo with ipsilateral turns represented in the positive direction and contralateral turns represented in the negative direction. Each point represents the mean number of turns per 5 min±S.E.M. Control (n=9), PGD_2 (n=4), PGE_2 (n=3), and $PGF_{2\alpha}$ (n=3). *$p<0.05$ when compared to controls at the same time period.

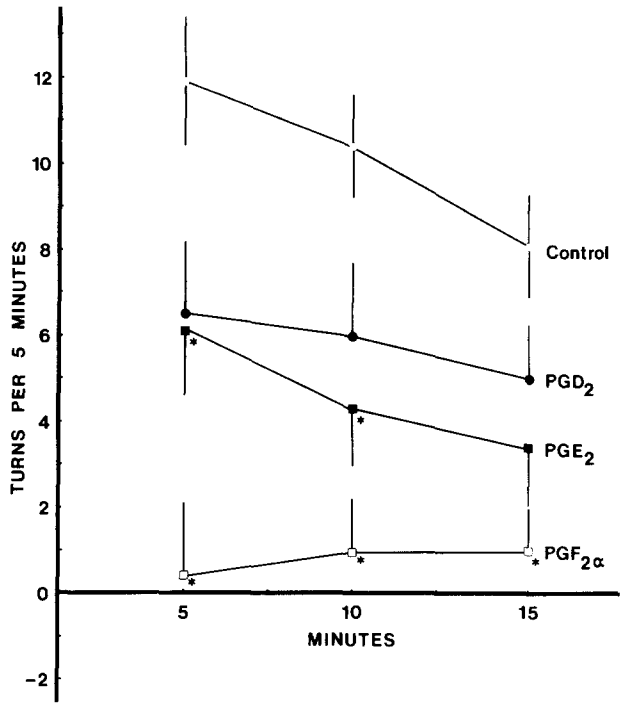

FIG. 2. Effect of intrastriatally injected prostaglandins on apomorphine-induced circling. Mice, previously electrolytically lesioned in the left striatum, were injected into the right striatum with saline or PGs (0.03 nmole/g) followed 10 min later by apo (1.0 mg/kg, IP). Counting of turns and direction are as in Fig. 1. Each point represents the mean number of turns per 5 min±S.E.M. Control (n=7), PGD_2 (n=4), PGE_2 (n=4), and $PGF_{2\alpha}$ (n=5). *$p<0.05$ when compared to controls at the same time period.

cover and then tested for a turning response in a manner similar to the 6-OHDA lesioned animals. Testing was performed 7 days after surgery.

Testing Protocol for Circling

The mice were injected centrally with either PG or saline and 10 min later administered apo (1.0 mg/kg, IP). The number of turns/5 min was counted beginning 5 min after the administration of apo. A "free hand" method similar to the one used by [12] was employed for the central injection of PG or saline into the brain. Under light, halothane anesthesia, mice were injected with PG or saline into the lateral ventricle (coordinates from bregma: 1.8 mm lateral and 2.5 mm below the surface of the skull) or the striatum (same coordinates as for the lesion, but drug was injected on the unlesioned side). All injections were made by means of a 10 μl Hamilton syringe, having a polyethylene cuff that allowed only the distal portion of the needle to be exposed. The 2 μl injections were made over a 45 sec period with the needle being held in place for an additional 15 sec. The incision was then closed with a wound clip, with the animals recovering from the anesthesia within 2–4 min after the injection. The site of the injection was verified at the end of the experiment by examining the location of dye after the injection of bromthymol blue into the same site as the PG injection. In all cases the dye was located at the proper site.

Statistics

Where appropriate, the data were analyzed by the Dunnett Test for comparing several means with a control mean or paired t-test.

RESULTS

Effect of Intraventricular Administration of Prostaglandins on Apomorphine-Induced Circling

Apo (1 mg/kg, IP) administration resulted in contralateral circling (away from the side of the lesion) in mice previously lesioned unilaterally with 6-OHDA in the left striatum. Five minutes after apo, mice injected with saline intraventricularly (controls) circled at a maximum rate of 25 turns/5 min and the circling remained relatively constant, showing only a small decrease for the next 15 min (Fig. 1). At a dose of 1.0 nmole/gm all PGs tested significantly inhibited apo-induced circling at 5 and 10 min when compared to saline control animals, while $PGF_{2\alpha}$ inhibited circling at all times examined (Fig. 1). PGs administered alone to 6-OHDA lesioned animals did not produce net circling in either direction (data not shown).

Effect of Intrastriatal Administration of Prostaglandins on Apomorphine-Induced Circling

Apo is thought to cause circling in unilateral 6-OHDA lesioned animals by acting on supersensitive DA receptors in the striatum. To determine whether PGs might act in the striatum to inhibit the effects of apo, we injected PGs or saline directly into the striatum followed by the administration of apo. In our preliminary studies we found that mice lesioned with 6-OHDA and then reinjected with saline into the same site as the lesion did not circle in response to apo.

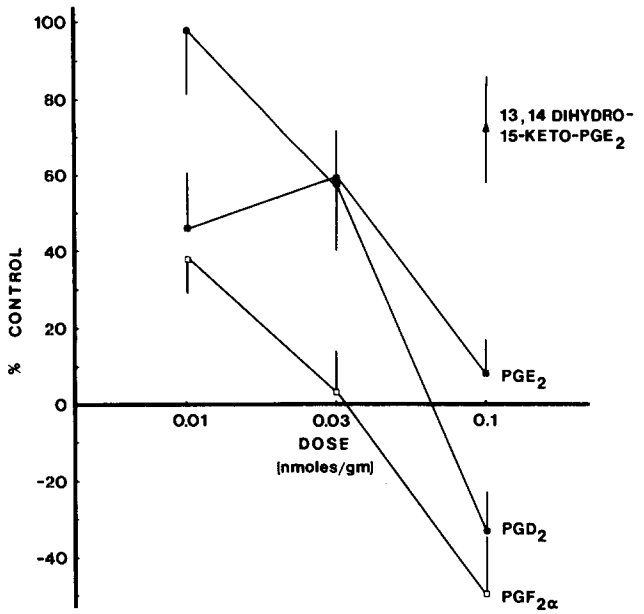

FIG. 3. Dose response of intrastriatally injected prostaglandins. The total number of turns in 15 min for each animal administered PG was calculated as percent of the total number of turns in 15 min of saline control animals. Each point represents the mean±S.E.M.

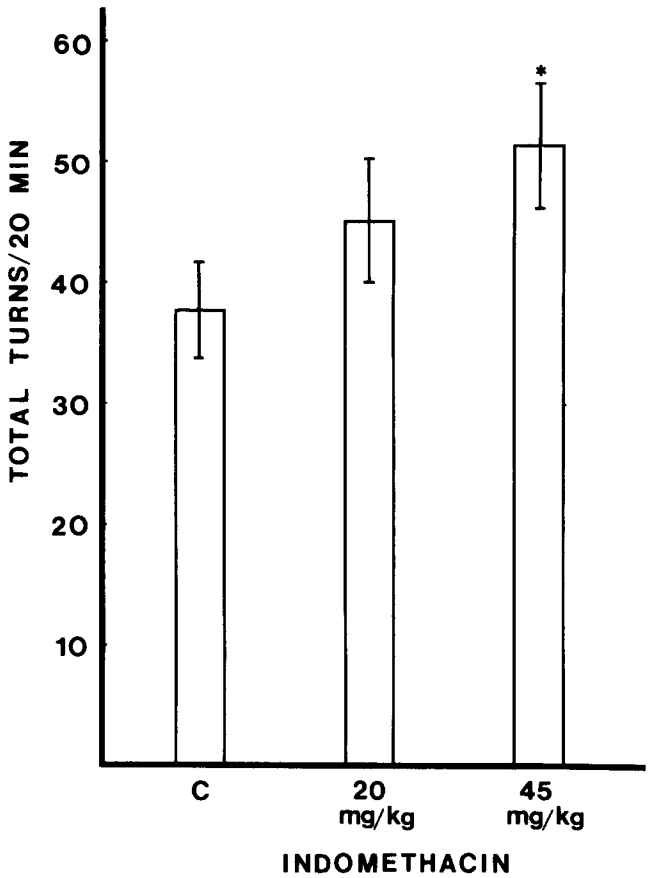

FIG. 4. Effect of indomethacin pretreatment on apomorphine-induced circling. Mice, previously electrolytically lesioned in the left striatum were pretreated with indomethacin (20 and 45 mg/kg, SC) 20 min prior to the administration of apo (1.0 mg/kg, IP). The number and direction of turns is as in Fig. 1. Each bar represents the mean total number of turns±S.E. of 8 animals. *$p<0.05$ when compared to control group.

Therefore, we were not able to use this procedure to determine the effect intrastriatally injected PGs. Consequently, mice were electrolytically lesioned in one striatum and PGs or saline were injected into the intact striatum before apo administration. After this lesion, pre- as well as postsynaptic elements are destroyed and the administration of apo systemically causes circling towards the side of the lesion (ipsilateral direction), since only DA receptors on the intact side are now available for stimulation. Figure 2 shows that animals injected intrastriatally with saline (control group) exhibit marked circling behavior after apo (1.0 mg/kg, IP). This circling was maximal 5 min after apo and thereafter decreased with time. Figure 2 also shows the effects of PGs over time at a dose of 0.03 nmole/g. $PGF_{2\alpha}$ produced a significant reduction in circling seen at the three time periods examined. PGE_2 inhibited circling at 5 and 10 min, while the effect of PGD_2 was not statistically different from saline control animals at any time period.

Figure 3 summarizes the effects of different doses of the three major PGs and 13,14-dihydro-15-keto-PGE_2 injected by the intrastriatal route of administration on apo-induced circling. In general, as the dose of PG was increased, there was a greater effect of these compounds on circling, with $PGF_{2\alpha}$ appearing to be the most potent of the PGs tested. At the highest dose of PG tested, 0.1 nmole/g, all of the PGs significantly decreased apo-induced circling. While PGE_2 almost completely blocked circling, $PGF_{2\alpha}$ and PGD_2 changed circling in the contralateral direction. In contrast, the PGE_2 metabolite, 13,14-dihydro-15-keto-PGE_2, produced a small decrease in circling, which was not significantly different from saline control animals.

Effect of Indomethacin Pretreatment Upon Apomorphine-Induced Circling

If endogenously synthesized PGs inhibit apo-induced circling, then the circling should be enhanced by indomethacin, an inhibitor of PG synthesis. The effect of a 20 min pretreatment with indomethacin on apo-induced circling was examined in mice electrolytically lesioned in the left striatum. As shown in Fig. 4, animals given 20 mg/kg indomethacin, IP, circled slightly, but not significantly more than control animals. However, animals given 45 mg/kg indomethacin circled at significantly higher rates than control animals.

DISCUSSION

Our initial observation that PGs inhibited amph-induced circling further indicated that PGs could affect DA-mediated neurotransmission [14,15]. The present results showing that PGs can inhibit apo-induced circling provide the first direct evidence showing that PGs can act at sites postsynaptic to the DA synapse within central DA-mediated pathways.

When injected intraventricularly, PGD_2, PGE_2, and $PGF_{2\alpha}$ all inhibited apo-induced circling at a dose of 1.0 nmole/g, with PGF_2 being the most potent. In order to determine whether the inhibition of circling could be due to the inhibition of the effects of DA receptor stimulation in the

striatum, PGs were injected intrastriatally. After intrastriatal injection all three major PGs tested inhibited apo-induced circling in the dose range of 0.01–0.1 nmole/g. In contrast the PGE_2 metabolite, 13,14-dihydro-15-keto-PGE_2, at a dose of 0.1 nmole/g, did not inhibit apo-induced circling suggesting that the inhibition of circling was not a characteristic effect of fatty acids derived from arachidonic acid. Similarly, in previous studies we have shown that the intrastriatal injection of 13,14-dihydro-15-keto-PGE_2 did not inhibit amph-induced circling at a dose in which the major PGs produced marked inhibition [14,15]. The doses of PGs that inhibited apo-induced circling were lower after intrastriatal injection than intraventricular injection. These results suggest that PGs inhibit apo-induced circling after intraventricular injection by acting in the striatum to inhibit the effects produced by DA receptor stimulation.

The administration of PGD_2 and $PGF_{2\alpha}$ at 0.1 nmole/g, the highest dose of PG administered intrastriatally, changed the direction of circling produced by apo in electrolytically lesioned animals, causing them to circle toward the intact side of the brain. Although the mechanism of this change in the direction of circling is not clear, circling behavior is generally thought to be due to a greater activity of neurons involved in motor function on one side of the brain compared to the other side [5]. After the electrolytic lesion of one striatum, net circling movements were not observed at 3 days following the lesion. This is presumably because neurons on both sides of the brain adjust to the unilateral lesion by balancing their activity. Apo by enhancing DA receptor stimulation on the intact side of the brain, may create an imbalance of neuronal activity and produce net circling toward the lesioned side of the brain. The PGs, when injected into the intact striatum inhibited the effects of DA receptor stimulation by apo as measured by the inhibition of circling behavior. It is possible that a high dose of PG may produce such a complete inhibition of the effects of DA receptor stimulation on the intact side, that an imbalance between the two sides is created so that activity of neurons involved in motor function on the lesioned side of the brain then becomes dominant. This could cause net circling behavior toward the intact side of the brain, and explain the change in direction of circling produced by high doses of PGs administered with apo.

The effectiveness of exogenously injected PGs in producing an inhibition of apo-induced circling suggests that endogenous PGs might function to inhibit the effects produced by DA receptor stimulation. Indomethacin has been shown to inhibit the enzyme, cyclooxygenase, which converts arachidonic acid to the endoperoxides (PGG_2 and PGH_2) which in turn are converted to the major PGs [4]. Although pretreatment with 20 mg/kg of indomethacin produced no significant change in apo-induced circling, a dose of 45 mg/kg, which has been used previously to inhibit PG formation in the brain [8], significantly increased the amount of circling seen after peripheral apo administration. These results support the hypothesis that endogenous PGs modulate motor function regulated by DA receptor stimulation.

The doses of the PGs injected intrastriatally required to inhibit apo-induced circling were similar to those previously shown to inhibit amph-induced circling. Unexpectedly, we found that although $PGF_{2\alpha}$ was the least potent of the PGs in inhibiting amph-induced circling, it was the most potent in inhibiting apo-induced circling. This difference may be due to a greater inhibitory effect of PGE_2 and PGD_2 on DA release induced by amph. Alternatively, these PGs might inhibit other effects of amph in the striatum that might contribute to the circling behavior induced by this drug, such as the release of serotonin from striatal nerve terminals. Stimulation of striatal serotonin receptors has been shown to produce ipsilateral circling in rats with unilateral destruction of nigral-striatal DA neurons [9,20]. Regardless of the interpretation, the greater effect of $PGF_{2\alpha}$ on apo-induced circling suggests that this compound may be more potent than the other PGs at sites postsynaptic to the DA synapse.

The inhibition of apo-induced circling could be caused by action of PGs that is not directly related to an action on neurons controlling motor function. One possibility is that alterations in body temperature produced by PGs is responsible for the inhibition. However, we have found that the marked inhibition of amph-induced circling by PGs occurred at a time when body temperature was not significantly different from saline control animals suggesting that the inhibition of circling is not related to a change in body temperature [15].

A second indirect mechanism through which PGs could act, is by a change in cerebral circulation. However, PGD_2 and PGE_2 are potent cerebral vasodilators [3], while $PGF_{2\alpha}$ causes vasoconstriction [21]. Since the behavioral changes produced by these PGs were in the same direction, changes in circulation would also be expected to occur in the same direction if this was the underlying mechanism. Thus, a change in cerebral blood flow does not appear responsible for the inhibition of apo-induced circling.

A third possible indirect mechanism by which PGs may alter behavior is by producing general CNS depression. Early studies [7], have shown that PGs injected intraventricularly produced catalepsy and a marked decrease in locomotion. Direct observation during our studies indicated that PGs injected intraventricularly at the dose of 1.0 nmole/g produced a small decrease in locomotor behavior, but the animals were not cataleptic. In addition, the doses injected intrastriatally neither decreased locomotor activity, not produced catelepsy (data not shown). Thus, it appears that a general depression of CNS function can not account for PG inhibition of apo circling.

Since our results suggest a postsynaptic site of PG alteration of DA neuronal mechanisms, further study into the exact site of action is warranted. It has been proposed that there may be multiple types of DA receptors [16]. Thus, PGs may act at the dopaminergic synapse or at synapses of non-dopaminergic neurons in the striatum which modulate the effects of DA receptor stimulation, such as cholinergic, gabergic, or enkephalinergic neurons.

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