Pharmacology Biochemistry & Behavior, Vol. 34, pp. 915–917. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

BRIEF COMMUNICATION

The Effect of Pre- and Postoperative Procedures on Physostigmine- and Apomorphine-Induced Yawning in Rats

ANNE BOURSON AND PAUL C. MOSER[1]

Merrell Dow Research Institute, 16 rue d'Ankara, 67084 Strasbourg Cedex, France

Received 20 March 1989

BOURSON, A. AND P. C. MOSER. *The effect of pre- and postoperative procedures on physostigmine- and apomorphine-induced yawning in rats*. PHARMACOL BIOCHEM BEHAV **34**(4) 915–917, 1989.—Previous experiments have shown that the potentiation of physostigmine-induced yawning by nifedipine is abolished by sham-lesioning procedures in rats, whereas the nifedipine potentiation of apomorphine-induced yawning is unaffected. The present results demonstrate that either the presurgical drug treatment (desmethylimipramine and pentobarbital) or 7 days isolation was alone sufficient to reduce the yawning response to physostigmine and abolish its potentiation by nifedipine. The sham-lesioned rats responded normally to a combination of apomorphine and nifedipine. These results suggest that the stress associated with standard operative procedures can differentially affect drug interactions with yawning induced by either apomorphine or physostigmine and that caution should be exercised when interpreting results from animals that have been similarly stressed.

Yawning Physostigmine Apomorphine Nifedipine Desmethylimipramine Pentobarbital Isolation stress Sham lesion

IN previous studies we have examined the effects of dihydropyridine (DHP) calcium channel blockers on yawning behavior in rats and have shown that they can potentiate both apomorphine- and physostigmine-induced yawning (1,2). In order to evaluate the site of this potentiation, part of these earlier studies investigated the effect of 6-hydroxydopamine lesions of the medial forebrain bundle (MFB) on the interaction between the DHP calcium channel blocker nifedipine and yawning induced by either apomorphine or physostigmine. We found that although sham-lesioned rats showed a normal nifedipine potentiation of apomorphine-induced yawning, yawning induced by physostigmine was no longer potentiated by nifedipine. This paper examines aspects of the operative procedure that might be responsible for this selective effect against physostigmine-induced yawning.

METHOD

Male Sprague-Dawley rats (Charles River, France), weighing 250–330 g, were used for all experiments. They were housed in wire cages in groups of 5 under controlled conditions of temperature (21 ± 1°C) and humidity (55% relative humidity) and under a 12-hr light-dark cycle with lights on between 06:00 and 18:00 hr. Rats were allowed free access to food and water.

Starting at least one week after their arrival from the suppliers, different groups of rats were subjected to various pretreatment schedules. One group of rats was treated approximately 30 min prior to surgery with desmethylimipramine (DMI, 25 mg/kg IP). The rats were then anesthetized with sodium pentobarbital (PB, 30 mg/kg IP, Clin-Midy, France) and placed in a stereotaxic frame (David Kopf Instruments). Bilateral sham lesions of the MFB were then carried out by slowly infusing 2 μl of sterile saline containing ascorbic acid (0.2 mg/ml) into the MFB at the following coordinates: AP = +3.8 mm, L = ± 1.5 mm (both with respect to bregma) and V = −8 mm from the dura (12). The infusions were made over a period of 5 min 30 sec and the injection cannula was left in place for a further 60 sec before being slowly withdrawn. Rats were subsequently housed individually and allowed to recover for a period of at least 7 days before use in yawning experiments as described below. Another group received PB and DMI pretreatment followed by 7 days isolation. Additional groups of rats received either only the drug pretreatment or saline, followed by group housing (5 rats per cage) and a further group, which received no injections, was housed singly for seven days prior to being used for yawning experiments. None of these groups underwent any actual surgical procedure.

[1]Requests for reprints should be addressed to Paul C. Moser.

TABLE 1

EFFECT OF NIFEDIPINE ON APOMORPHINE- AND PHYSOSTIGMINE-INDUCED YAWNING IN SHAM-LESIONED RATS

Treatment (μg/kg SC)	Pretreatment (mg/kg IP)	n	Yawns/30 Min (mean ± SEM)
Apomorphine (40)	PEG 50%	8	4.8 ± 1.3
	Nifedipine (10)	8	12.6 ± 2.9*
Physostigmine (50)	PEG 50%	8	1.1 ± 0.7
	Nifedipine (10)	8	1.8 ± 0.6

*$p<0.05$ compared to PEG 50%-treated rats (Mann-Whitney U-test).

Yawning Experiments

Nifedipine (10 mg/kg IP) or 50% polyethyleneglycol (PEG 50%) was administered 30 min before injection of apomorphine (40 μg/kg SC) or physostigmine (50 μg/kg SC). Immediately following the injection of apomorphine or physostigmine, rats were individually placed in clear glass beakers (18 cm high × 14.5 cm diameter) containing a layer of sawdust with a mirror behind the beakers to allow all round observation of the rats. The number of yawns was counted by direct observation for a period of 30 min from the time that the rats were placed in the beakers. All yawning experiments were carried out between 08:30 and 12:30 hr and each rat was used only once.

Drugs and Statistics

Apomorphine and physostigmine were dissolved in saline (0.9% w/v NaCl) and nifedipine in 50% polyethyleneglycol (PEG 50%). All drug solutions were freshly prepared and solutions of apomorphine and nifedipine were protected from light. All doses refer to the salt where appropriate. Apomorphine HCl, desmethylimipramine HCl, physostigmine and nifedipine were obtained from Sigma (USA). Statistical analyses of differences between control and test groups were carried out with a two-tailed Mann-Whitney U-test and were considered as significant if $p<0.05$.

RESULTS

Effect of Nifedipine on Yawning in Sham-Lesioned Rats

In three prior experiments using normal rats which had been pretreated with PEG 50% (2), apomorphine (40 μg/kg SC) and physostigmine (50 μg/kg SC) produced 7.7±0.7 and 6.0±0.9 yawns/30 min (mean±SEM, n=32), respectively. Both compounds also induced yawning in sham-lesioned rats, although the responses were significantly less than those observed in normal rats ($p<0.05$ and $p<0.01$ for apomorphine and physostigmine respectively using the Mann-Whitney U-test). Pretreatment with nifedipine (10 mg/kg IP) significantly potentiated this response to apomorphine, but not to physostigmine (Table 1).

Influence of Isolation and Drug Pretreatment

Table 2 presents the results of several experiments designed to assess the role of different components of the surgical procedure in the effects observed in sham-lesioned rats. Pretreatment with DMI and PB in combination with a period of 7 days isolation was sufficient, without any surgical procedure, to prevent the potentiation of physostigmine-induced yawning by nifedipine (Table 2A). Similarly, isolation for 7 days was also found to be sufficient, on its own, to prevent the potentiation of physostigmine-induced yawning by nifedipine compared to rats housed in groups of 5 (Table 2B), as was pretreatment with DMI and PB in group-housed rats 7 days before the yawning experiment (Table 2C). Injections of saline 7 days before had no such inhibitory effect on the nifedipine potentiation of physostigmine in group-housed rats.

TABLE 2

EFFECT OF ISOLATION AND PRETREATMENT WITH BOTH DMI (25 mg/kg IP) AND PB (30 mg/kg IP) ON THE NIFEDIPINE POTENTIATION OF PHYSOSTIGMINE-INDUCED YAWNING IN RATS NOT UNDERGOING STEREOTAXIC SURGERY

	Pretreatment	Housing Condition	n	Yawns/30 Min (mean ± SEM) PEG 50%	Nifedipine (10 mg/kg IP)
A	DMI + PB	Isolation	5	3.2 ± 1.4	3.0 ± 2.1
B	None	Isolation	5	0.4 ± 0.2	1.4 ± 0.9
	None	Group	5	1.6 ± 0.9	10.6 ± 3.0*
C	Saline	Group	10	5.4 ± 1.3	11.8 ± 2.3*
	DMI + PB	Group	5	4.8 ± 1.5	4.3 ± 1.3

*$p<0.05$ compared to PEG 50%-treated rats (Mann-Whitney U-test).

DISCUSSION

These results show that not only the sham operation procedure, but also 7 days isolation or pretreatment with DMI and PB, can prevent the nifedipine potentiation of physostigmine-induced yawning. In this respect, the yawning response to physostigmine differs from that to apomorphine, which was still potentiated by nifedipine after the complete sham operation procedure (Table 1), suggesting that individual components of the sham operation procedure would also be ineffective in preventing the effect of nifedipine.

We consider that the effects we have described are due to the stress associated with the procedures used. It is well established that isolation is a stressful procedure which can affect many behavioral and physiological parameters in rats (3,9), and it has recently been demonstrated that 28 days isolation can significantly attenuate the yawning response to dopamine agonists (5). Although the yawning response to both physostigmine and apomorphine was reduced by the sham operation procedure, this effect was particularly marked for physostigmine, consistent with previous suggestions that cholinergic agonist-induced yawning is more susceptible to modulation by stress than that induced by dopamine agonists (7). In addition, sham-lesioning abolished the nifedipine-potentiation of physostigmine-induced yawning behavior, an effect mimicked by either isolation for seven days or pretreatment with DMI and PB. This suggests that the interaction of nifedipine with physostigmine-induced yawning is even more susceptible to modulation by stress than yawning behaviour itself.

The results obtained with DMI and PB pretreatment may be due to the stress associated with anesthesia. The combination of DMI and PB was used as this was part of the original protocol for sham-lesioning, the DMI being included to prevent the uptake of 6-OHDA into noradrenergic terminals in lesioned rats. Although it is unlikely that the drug effects themselves would have lasted 7 days, the injection of physostigmine on the test day may have induced a conditioned stress response, similar to the conditioned emotional response (4). Further evidence that this is not a direct effect of drug treatment comes from the demonstration that DMI

will itself produce yawning in rats (11).

As many studies suggest that apomorphine-induced yawning is mediated via the cholinergic system (10,13), the differences between apomorphine- and physostigmine-induced yawning were unexpected, and it is not clear at present why mildly stressful events should selectively affect the interaction of nifedipine with physostigmine. The dose-response curves for both apomorphine- and physostigmine-induced yawning follow an inverted U-shape, and while the apomorphine dose-response curve can be explained by pre- and postsynaptic effects (8), that of physostigmine is due to secondary drug effects, such as chewing and behavioral arousal, which prevent the appearance of yawning. It is possible that stressful events make rats more sensitive to such secondary effects, leading to a suppression of yawning. If these inhibitory effects are sufficiently strong, treatment with nifedipine may not be able to increase yawning. The observations that stress can markedly affect neurochemical parameters associated with cholinergic function (6) would support such a proposal, although further work is needed to determine the manner in which stress changes the yawning response to physostigmine. It should be noted that other authors have remarked on the greater variability of physostigmine-induced yawning compared to apomorphine-induced yawning, particularly between groups, and have suggested that this effect of physostigmine is more susceptible to external influence (7).

In conclusion, these results show that relatively mild stress can affect physostigmine-induced yawning in both a quantitative manner (reducing the number of yawns) and a qualitative manner (preventing its interaction with nifedipine). Both these effects appear to be specific for physostigmine-induced yawning, as even when these procedures were combined in the sham operation they only slightly reduced apomorphine-induced yawning and did not prevent its interaction with nifedipine. It is clear that special attention towards housing conditions and preinjection routines is needed when studying yawning behavior, and that such sources of variation must be eliminated before accurate conclusions can be drawn from the results. In the present case, for example, results from stressed rats would suggest that dihydropyridine calcium channel blockers have a selective interaction with apomorphine-induced yawning, whereas, in fact, they interact with both physostigmine- and apomorphine-induced yawning and the site of this interaction is more likely to be associated with cholinergic than dopaminergic neurones (2).

REFERENCES

1. Bourson, A.; Gower, A. J.; Mir, A. K. Central biochemical and behavioral effects of BAY K 8644 in the rat. Br. J. Pharmacol. 89:625P; 1986.
2. Bourson, A.; Moser, P. C. Potentiation by dihydropyridine calcium channel blockers of physostigmine-induced yawning in rats. Br. J. Pharmacol. 97:468P; 1989.
3. Brain, P.; Benton, D. The interpretation of physiological correlates of differential housing in laboratory rats. Life Sci. 24:99–116; 1979.
4. Estes, W. K.; Skinner, B. F. Some quantitative properties of anxiety. J. Exp. Psychol. 29:390–400; 1941.
5. Ferrari, F.; Mangiafico, V. Effect of isolation on dopaminergic agonist-induced penile erections and stretching and yawning in male rats. Behav. Brain Res. 28:309–313; 1988.
6. Gilad, G. M.; Mahon, B. D.; Finkelstein, Y.; Koffler, B.; Gilad, V. H. Stress-induced activation of the hippocampal cholinergic system and the pituitary-adrenocortical axis. Brain Res. 347:404–408; 1985.
7. Gower, A. J. Effects of acetylcholine agonists and antagonists on yawning and analgesia in the rat. Eur. J. Pharmacol. 139:79–89; 1987.
8. Gower, A. J.; Berendsen, H. H. G.; Princen, M. M.; Broekkamp, C. L. E. The yawning-penile erection syndrome as a model for putative dopamine autoreceptor activity. Eur. J. Pharmacol. 103:81–89; 1984.
9. Hatch, A. M.; Wiberg, G. S.; Zawidzka, Z.; Cann, M.; Airth, J. M.; Grice, H. C. Isolation syndrome in the rat. Toxicol. Appl. Pharmacol. 7:737–745; 1965.
10. Holmgren, B.; Urba-Holmgren, R. Interaction of cholinergic and dopaminergic influences on yawning behavior. Acta Neurobiol. Exp. 40:633–642; 1980.
11. Mogilnicka, E.; Wedzony, K.; Klimek, V.; Czyrak, A. Desipramine induces yawning behavior in rats. Neuropharmacology 25:783–786; 1986.
12. Pellegrino, L. J.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Appleton-Century Crofts; 1967.
13. Yamada, K.; Furukawa, T. Direct evidence for involvement of dopaminergic inhibition and cholinergic activation in yawning. Psychopharmacology (Berlin) 67:39–43; 1980.