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Atropine Increases Pilocarpine-Induced Yawning Behavior in Paradoxical Sleep Deprived Rats

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LOBO, L. L., R. DE MEDEIROS, D. C. HIPÓLIDE AND S. TUFIK. *Atropine increases pilocarpine-induced yawning behavior in paradoxical sleep deprived rats.* PHARMACOL BIOCHEM BEHAV **52**(3) 485–488, 1995.—Paradoxical sleep (PS) deprivation has been suggested to induce supersensitivity of postsynaptic dopamine (DA) receptors and subsensitivity of acetylcholine (ACh) receptors. Yawning behavior is reduced after PS deprivation and is believed to result from an interaction between ACh and DA systems. Concomitant treatment of PS deprived animals with DA agonists reverses PS deprivation effects on stereotypy and aggressiveness. To examine this possibility on yawning behavior, rats were treated, during the deprivation period, with atropine, methamphetamine, haloperidol or distilled water. Following PS deprivation, rats were injected with apomorphine or pilocarpine and number of yawns was recorded. Atropine increased yawning of PS deprived rats induced by pilocarpine, but not by apomorphine. Treatment with methamphetamine and haloperidol did not change PS deprivation effect on pilocarpine- and apomorphine-induced yawning. The data suggest that reversal of PS deprivation-induced yawning inhibition is mediated distinctly by both acetylcholine and dopamine systems.

Paradoxical sleep deprivation Yawning Atropine Methamphetamine Haloperidol Pilocarpine Apomorphine Rats

THE FUNCTIONAL significance of yawning is still unknown, although this behavior has been studied for the last 3 to 4 decades. Yawning can be elicited by several cholinergic (AChergic) agonists (28,32), by low doses of dopaminergic (DAergic) agonists (11), and by polypeptides such as α-MSH and ACTH (2,10,33). Moreover, Gower et al. (4) showed that serotonin, histamine, and noradrenaline systems play a role in modulating this behavior.

Yawning has been suggested to result from a balance between DAergic and AChergic systems. This conclusion is based on a study by Yamada and Furukawa (32), who showed that scopolamine, a cholinoceptor antagonist, inhibits apomorphine-, pilocarpine-, and physostigmine-induced yawning. The neuroleptic fluphenazine, however, does not modify pilocarpine-induced, but increases the number of physostigmine-induced yawning. In addition, sulpiride, a D_2 dopamine (DA) receptor blocker, inhibits apomorphine-induced, but not physostigmine-induced yawning (4). Thus, yawning appears to be mediated by inhibition of DAergic system and activation of AChergic system.

Paradoxical sleep (PS) deprivation induces several changes on human's and rat's behavior, such as increased apomorphine-induced stereotypy and aggressive behavior in rats (26), and improvement of endogenous depression symptoms in humans (9,17,23,31). It is proposed that these effects are a consequence of postsynaptic DA receptors supersensitivity (26), and presynaptic DA receptors and postsynaptic acetylcholine (ACh) receptors subsensitivity (27). PS deprivation also inhibits its yawning elicited by apomorphine, physostigmine, and pilocarpine (27).

Haracz and Tseng (6) showed that DA receptor supersensitivity resulting from neuroleptic treatment is attenuated by an acute dose of amphetamine. In addition, treatment of PS deprived animals with amphetamine and L-dopa results in re-

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versal of deprivation effects on apomorphine-induced stereotypy and aggressiveness; behaviors that occur as a consequence of postsynaptic DA receptor supersensitivity (24).

Because PS deprivation produces an ACh receptor subsensitivity, as well as a DA receptor supersensitivity, the evaluation of yawning behavior in PS deprived animals seemed adequate to better understand the postulated relationship between these neurotransmitter systems. Thus, in the present study, we tested whether manipulations on these systems would result in change of PS deprivation induced inhibition of yawning behavior.

METHODS

Subjects

Male Wistar rats from our own colony, weighing 250–340 g and kept in groups of three in wire mesh cages, were used. Animals were kept in a room with controlled temperature (23° ± 1°C) and 12 h light/dark cycle (lights on 0700 h). Food and water were, at all moments, including the deprivation period, available ad lib.

Drugs

Apomorphine hydrochloride (DAergic agonist), atropine sulfate (muscarinic antagonist), methamphetamine (catecholaminergic agonist), pilocarpine hydrochloride (AChergic agonist) (Sigma Chemical Co., St. Louis, MO) and haloperidol (DAergic antagonist) (Janssen Pharm., Brazil) were used. Drugs were dissolved in distilled water and given in a volume of 1 ml/kg of body weight. All drugs were administered IP, except apomorphine, which was administered SC.

PS Deprivation

Rats were PS deprived for 96 h by the multiple platform technique, an adaptation of the water tank technique (7,12). This technique prevents the stresses of immobilization and social isolation that accompanies the classical procedure (15). Deprivation was performed by placing ten animals in a large water tank (125 × 45 × 36 cm), containing 17 inverted platforms (6 cm in diameter), placed approximately 10-cm apart. The tank was filled with water to a level of 1-cm below the platforms' surface. Water in the tank was changed daily.

Treatment Procedure

PS deprived rats were assigned to one of five subgroups: (a) atropine (2.0 mg/kg); (b) methamphetamine (2.0 mg/kg); (c) methamphetamine (5.0 mg/kg); (d) haloperidol (0.5 mg/kg); and (e) haloperidol (1.0 mg/kg). Each one of the drug-injected groups was paired to a corresponding control group, which consisted of animals treated with distilled water during PS deprivation. Rats were treated daily at 1400 h, during the deprivation period. The fourth and last injection was given 24 h before testing.

Testing Procedure

Immediately after the end of deprivation, animals (atropine: 20/dose of each testing drug; methamphetamine or haloperidol: 10/dose of each testing drug; number of water-treated animals was the same as for each of the drug-treated groups) were injected with one of three doses of either apomorphine (20, 40, and 80 μg/kg) or pilocarpine (1.0, 2.0, and 4.0 mg/kg) and placed, individually, in wire mesh cages. The doses chosen were based on previous studies (14,27). Number of yawns was recorded during 30 min. Testing took place between 1400 and 1530 h.

Statistical Analysis

Comparison between drug-treated and distilled water-treated groups was made by Mann-Whitney's *U*-test, with the level of significance set at $p < 0.05$ (two-tailed).

RESULTS

Atropine Treatment

As shown in Fig. 1A, atropine treatment did not modify apomorphine-induced yawning, whereas it increased pilocarpine (1.0 and 2.0 mg/kg) induced yawning of PS deprived animals (Fig. 1B).

Neither methamphetamine nor haloperidol treatments resulted in change of yawning behavior of PS deprived rats induced by pilocarpine or apomorphine.

DISCUSSION

Induction of yawning behavior appears to be dependent on a balance between DAergic and AChergic systems. This does not seem to be the case for reversal of yawning inhibition resulting from PS deprivation. Our results showed that during the deprivation period only treatment with an AChergic antagonist was effective in counteracting PS deprivation effects on pilocarpine-induced yawning. Atropine effect was quite specific, because it did not reverse apomorphine-induced yawning. Furthermore, neither DAergic agonist nor antagonist affected PS deprivation induced inhibition of yawning. This result suggests the DAergic system involved with this behavior is different from that involved with stereotypy and aggressive-

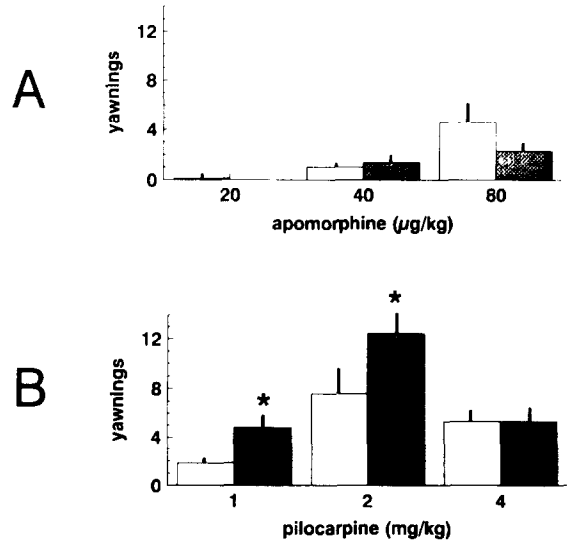

FIG. 1. Effects of treatment with 2.0 mg/kg of atropine during PS deprivation on yawning induced by (A) apomorphine (n = 20/group per dose) and (B) pilocarpine (n = 20/group per dose). Values are represented by mean ± SE of control (open bars) and experimental (hatched bars) groups.

ness, because pretreatment with amphetamine and L-dopa reversed PS deprivation effects on these behaviors (24).

Yawning is elicited, among several other manipulations, by activation of postsynaptic ACh receptors or presynaptic DA receptors, suggesting a DA-ACh link mediating this behavior (32), via DA inhibition and, consequently, ACh activation. This hypothesis was further supported by several studies (4,29), although it is still not clear which receptors are responsible for this behavior (3,20). There is a controversy about the mechanisms by which yawning is elicited. Several authors suggest yawning is mediated by stimulation of presynaptic DAergic receptors (11,32). On contrary, Serra et al. (18,19) propose that this behavior is elicited by stimulation of a special population of postsynaptic DA receptors, and D_1 and D_2 receptors' supersensitivity would inhibit this special receptor. Thus, studies using amphetamine pretreatment do not result in inhibition of yawning induced by low doses of apomorphine, leading the authors to suggest that D_2 presynaptic receptors are not the mediators of yawning behavior (21). In addition to this hypothesis, behavioral data suggest yawning as a D_3-dependent phenomenon (8). Results from biochemical and molecular cloning techniques support the idea of DA receptor subtypes other than the classical D_1 and D_2 receptors described (1).

Inhibition of pilocarpine-, physostigmine-, and apomorphine-induced yawning is observed following PS deprivation (27). This manipulation is believed to inhibit DAergic transmission, leading to increased AChergic transmission. Supposedly, the systems respond to PS deprivation with a presynaptic DA and a postsynaptic ACh receptor subsensitivity (16,27). Cholinergic system appears to have an important role in mediating PS: M_2 ACh receptor agonists trigger this sleep stage (30). Moreover, an autoradiographic study in 96-h PS deprived animals reveals a M_2 ACh receptor downregulation (16), suggesting increased ACh transmission during PS deprivation. Thus, atropine treatment during deprivation could antagonize the suggested subsensitivity of AChergic receptors induced by PS deprivation (27). Recently, Szymusiak et al. (22) reported reversal of atropine-induced PS inhibition at high (30°C) ambient temperature. Supposedly, both high temperature (22) and PS deprivation (16,27), induce an increase in ACh neurotransmission.

Low doses of haloperidol block presynaptic DA receptors, and this effect should prevent DA receptor subsensitivity induced by PS deprivation. The lack of haloperidol effect, observed in this study, could be a consequence of treatment schedule; it is possible that daily injections during 4 days was not long enough to alter receptor sensitivity (13). Tufik (25), however, observed changes in receptor sensitivity following acute treatment with haloperidol. Similarly, Greenshaw et al. (5) referred to several studies demonstrating short-term neuroleptic-induced increase in DA receptor binding. In addition, the hypothesis that normalization of dopaminergic supersensitivity as a result of methamphetamine treatment during PS deprivation would restore the frequency of yawns was not corroborated by our findings. Therefore, these data reveal that classical manipulations of dopaminergic system, such as methamphetamine and haloperidol treatments, had no effect on yawning frequency of PS deprived rats. DA system appears to be affected sooner by PS deprivation, and to recover later than the ACh system, suggesting that PS deprivation-induced changes on these systems occur at different moments (10,14). It is possible that reversal of yawning inhibition induced by PS deprivation also occurs sooner for AChergic than DAergic system. Our results support this hypothesis and also suggest an independent mediation of both systems on reversal of PS deprivation-induced yawning inhibition.

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REFERENCES

1. Andersen, P. H.; Gingrich, J. A.; Bates, M. D.; Dearry, A.; Falardeau, P.; Serrogles, S. E.; Caron, M. G. Dopamine receptor subtypes: Beyond the D_1/D_2 classification. Trends Pharmacol. Sci. 11:231-236; 1990.
2. Ferrari, W.; Gessa, G. L.; Vargiu, L. Behavioral effects induced by intracisternally injected ACTH and MSH. Ann. N. Y. Acad. Sci. 104:330-345; 1963.
3. Gower, A. J. Effects of acetylcholine agonists and antagonists on yawning and analgesia in the rat. Eur. J. Pharmacol. 139:79-80; 1987.
4. Gower, A. J.; Beredsen, N. N. G.; Broekkamp, C. L. Antagonism of drug induced-yawning and penile erection in rats. Eur. J. Pharmacol. 122:239-244; 1986.
5. Greenshaw, A. J.; Baker, G. B.; Wishart, T. B. Dopamine receptor changes during chronic drug administration. In: Goudie, A. J.; Emmett-Oglesby, M. W., eds. Psychoactive drugs: Tolerance and sensitization. New Jersey: Humana Press; 1989:353-406.
6. Haracz, J.; Tseng, L. F. Reduction of dopaminergic supersensitivity by a single dose of amphetamine. Naunyn-Schmiedeberg's Arch. Pharmacol. 313:131-133; 1980.
7. Jouvet, D.; Vimont, E.; Delorme, F.; Jouvet, M. Étude de la privation sélective de la phase paradoxale de sommeil chez le chat. C. R. Soc. Biol. 158: 756-759; 1964.
8. Kostrzewa, R. M.; Brus, R. Is dopamine-agonist induced yawning behavior a D_3 mediated event? Life Sci. 48:PL-129; 1991.
9. Kuhs, H.; Tolle, R. Sleep deprivation therapy. Biol. Psychiatry 29:1129-1148; 1991.
10. Lobo, L. L.; Neumann, B. G.; Eidman, D. S.; Tufik, S. Effects of REM sleep deprivation on ACTH-induced yawning. Pharmacology 40:174-178; 1990.
11. Mogilnicka, E.; Klimek, V. Drugs affecting dopamine neurons and yawning behavior. Pharmacol. Biochem. Behav. 7:303-305; 1977.
12. Morden, B.; Mitchell, G.; Dement, W. C. Selective REM sleep deprivation and compensation phenomena in the rat. Brain Res. 5:339-349; 1967.
13. Muller, P.; Seeman, P. Brain neurotransmitter receptors after long-term haloperidol: Dopamine, acetylcholine, serotonin, noradrenergic and naloxone receptors. Life Sci. 21:1751-1758; 1977.
14. Neumann, B. G.; Troncone, L. R. P.; Braz, S.; Tufik, S. Modifications on dopaminergic and cholinergic systems induced by the water tank technique: Analysis through yawning behavior. Arch. Int. Pharmacodyn. Ther. 308:32-38; 1990.
15. Nunes, G.P., Jr.; Tufik, S. Validation of the modified multiple platform method (MPM) of paradoxical sleep deprivation in rats. Sleep Res. 23:419; 1994.

16. Nunes, G.P., Jr.; Tufik, S.; Nobrega, J. N. Autoradiographic study of dopaminergic and cholinergic receptors after paradoxical sleep deprivation. Sleep Res. 22:339; 1993.
17. Schittecatte, M.; Charles, G.; Machowski, R.; Valentin, J. G.; Mendlewicz, J.; Wilmotte, J. Reduced clonidine rapid eye movement sleep suppression in patients with primary major affective illness. Arch. Gen. Psychiat. 49:637-642; 1992.
18. Serra, G.; Collu, M.; Gessa, G. L. Dopamine receptors mediating yawning: Are they autoreceptors? Eur. J. Pharmacol. 120:187-192; 1986.
19. Serra, G.; Collu, M.; Gessa, G. L.; Ferrari, W. Melanocortine and dopamine link yawning behavior. In: De Wied, D.; Ferrari, W., eds. Central actions of ACTH and related peptides. Fidia Research Series, Symposia in Neuroscience, Vol 4. Padova; 1986: 163-178.
20. Stahle, L. Do autoreceptors mediate dopamine agonist-induced yawning and suppression of exploration? A critical review. Psychopharmacology 106:1-13; 1992.
21. Stahle, L.; Ungerstedt, U. Yawning and suppression of exploration in amphetamine-treated rats, incompatibility with the autoreceptor hypothesis. Psychopharmacology 97:553-560; 1989.
22. Szymusiak, R.; Danowski, J.; McGinty, D. REM sleep-suppressing effects of atropine in cats vary with environmental temperature. Brain Res. 636:115-118; 1994.
23. Szuba, M. P.; Baxter, L. R., Jr.; Fairbanks, L. A.; Guze, B. H.; Schwart, J. M. Effects of partial sleep deprivation on the diurnal variation of mood and motor activity in major depression. Biol. Psychiat. 30:817-829; 1991.
24. Troncone, L. R. P.; Ferreira, T. M. S.; Braz, S.; Silveira Filho, N. G.; Tufik, S. Reversal of the increase in apomorphine-induced stereotypy and aggression in REM sleep deprived rats by dopamine agonist pretreatments. Psychopharmacology 94:79-83; 1988.
25. Tufik, S. Changes of response to dopaminergic drugs in rats submitted to REM sleep deprivation. Psychopharmacology 72:257-260; 1981.
26. Tufik, S.; Lindsey, C. J.; Carlini, E. A. Does REM sleep deprivation induce a supersensitivity of dopaminergic receptors in the rat brain? Pharmacology 16:98-105; 1978.
27. Tufik, S.; Troncone, L. R. P.; Braz, S.; Silva-Filho, A. R.; Neumann, B.G. Does REM sleep deprivation induce subsensitivity of presynaptic dopamine or postsynaptic acetylcholine receptors in the rat brain? Eur. J. Pharmacol. 140:215-219; 1987.
28. Urba-Holmgren, R.; Gonzalez, R. M.; Holmgren, B. Is yawning a cholinergic response? Nature 267:261-262; 1977.
29. Ushijima, I.; Mizuki, Y.; Yamada, M. Multifocal sites of action involved in dopaminergic-cholinergic neuronal interaction in yawning. Psychopharmacology 95:34-37; 1988.
30. Velazquez-Moctezuma, J.; Gillin, J. C.; Shiromani, P. J. Effect of specific M_1, M_2 muscarinic receptor agonists on REM sleep generation. Brain Res. 503:128-131; 1989.
31. Vogel, G. W.; Thurmond, A.; Gibbons, P.; Sloan, K.; Boyd, M.; Walher, M. REM sleep reduction effects on depression syndromes. Arch. Gen. Psychiat. 32:765-777; 1975.
32. Yamada, K.; Furukawa, T. Direct evidence for involvement of dopaminergic inhibition and cholinergic activation in yawning. Psychopharmacology 67:39-43; 1980.
33. Yamada, K.; Furukawa, T. The yawning elicited by α-melanocyte stimulating hormone involves serotonergic-dopaminergic-cholinergic neuron link in rats. Naunyn-Schmiedeberg's Arch. Pharmacol. 316:155-160; 1981.