Behavioural Brain Research, 56 (1993) 155–159

BBR 01479

Two inbred rat sublines that differ in spontaneous yawning behavior also differ in their responses to cholinergic and dopaminergic drugs

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(Received 24 July 1992)
(Revised version received 6 April 1993)
(Accepted 12 May 1993)

Key words: Rat behavior; Genetics; Yawning pharmacology

This work compares the sensitivities of high-yawning (HY) and low-yawning (LY) sublines of Sprague–Dawley rats to dopaminergic and cholinergic yawning-inducing drugs. HY animals are significantly more sensitive to apomorphine and (−)3PPP than LY animals. Physostigmine is a less effective yawning-inducer in HY than in LY rats. With pilocarpine no differences were detected between both sublines in regard to its yawning-inducing activity. Since yawning behavior is subject to dopaminergic (inhibitory) and cholinergic (excitatory) influences, it is suggested that the genetic differences between these sublines affect the dopaminergic pathways that normally regulate yawning frequency.

INTRODUCTION

Yawning is a behavioral pattern that has gained increasing recognition in this last quarter of the twentieth century. The easiness with which this motor act may be induced, or modulated in frequency by different pharmacological agents in laboratory animals, particularly among rodents, has brought to focus a complex set of neurotransmitters and hormonal mechanisms underlying yawning behavior[3].

It has been suggested that regulation of yawning is partly the result of an interaction between dopaminergic and cholinergic influences on the central yawning pattern generator[3]. The fine details and localization of these interactions in the central nervous system have not been entirely worked out. Some authors[16,18] believe that dopaminergic induction of yawning results from activation of low threshold postsynaptic receptors exciting yawning, while others[4,6,8,14,15,22,28,30] suggest it is due to D_2 autoreceptors restraining the dopaminergic inhibitory influences on yawning, thus disinhibiting cholinergic yawn-excitatory pathways. There is practically general agreement in that muscarinic cholinergic antagonists counteract yawning, whatever the pharmacological tool used to induce it (ACTH[7], physostigmine or pilocarpine[21], apomorphine or other dopamine agonists[10,12,13,25,30]). These results suggest that the cholinergic link in yawning regulation is downstream in relation to the dopaminergic one.

In a previous paper in this same journal[23] we reported the development of two inbred sublines of Sprague–Dawley rats which differ significantly in spontaneous mean yawning frequency (MYF): one of them yawns at a low frequency (LY); the other at a higher rate (HY). The present report is an attempt to identify the neural substrates of this genetic difference in spontaneous yawning frequency between HY and LY Sprague–Dawley rats, by comparing their sensitivities to well known dopaminergic and cholinergic yawn-inducing drugs.

MATERIALS AND METHODS

Animals and behavioral observations

The experimental subjects were LY and HY Sprague–Dawley male rats, 60–75 days old. The animals were weaned at age of 1 month, and housed under standard conditions, as previously described[23]. On the day of behavioral testing rats were moved to a quiet well lit laboratory room and allowed to habituate to their new environment for 1 h before yawn monitoring began. Observations were performed with each rat placed in a transparent glass cylinder (diameter 190 mm, height

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100 mm), the floor of which was covered with a sheet of clean filter paper and the top with a plexiglass plate, leaving a 1 cm wide segment open for ventilation. Observations were done regularly between 09.00 and 10.00 h to minimize circadian variation[1]. Yawning was monitored by two trained observers (one of them performing a blind experiment) sitting on opposite sides of the table on which the animals were placed. A yawn was scored when a rat opened its mouth wide and gradually, retained the opened position during a couple of seconds, and closed the mouth rapidly. The movement was usually (but not always) accompanied by extension of the neck and one or both forelimbs. The results are expressed as frequencies (number of yawns/time). Animals, from both sublines, were selected at random for paired observations and used only once.

Drugs

The following drugs were used: physostigmine sulfate (Sigma, USA); pilocarpine·HCl (Sigma, USA), (−)3-PPP (3-(3 hydroxy phenyl)-*N*-*n*-propylpiperidine), Astra, Sweden) and Apomorphine·HCl (Sigma, USA).

Solutions were freshly prepared in distilled water and further diluted with saline to reach a standard injection volume of 2 ml/kg bodyweight. Apomorphine (APO) was dissolved in water containing ascorbic acid (0.2 mg/ml) to hinder oxidation. Doses are expressed in terms of the free base. All drugs, except APO, were administered intraperitoneally. APO was injected subcutaneously in the lumbar region. Controls received corresponding vehicle injections. (−)3PPP was administered 15 min before observations began. The rest of the drugs were injected immediately before observation.

Statistics

The behavioral dose–response curves were first analyzed by using the Kruskal–Wallis test (KWT) of variance followed by individual comparisons between drug-treated and control groups with the Mann–Whitney U test (MWUT)[19]. Since LY and HY rats basal MYF's are so different, in order to compare drug-induced effects in both sublines, the absolute (additional) number of yawns induced by the drugs were calculated. With data so obtained we traced dose-response curves, with the ascending part of which a linear regression analysis was performed. The ED_{50}s were estimated by interpolation. LY and HY regression lines were tested for parallelism by use of T distribution as recommended by Tallarida and Murray[20]. In all cases the criterion for statistical significance was estimated at $P<0.05$.

RESULTS

Yawn-induction by cholinergic drugs

1. Physostigmine (PHY) effects

The yawning responses to different doses of PHY in HY (F21) and LY (F18–19) rats are shown in Fig. 1. Both curves are quite similar in shape, but higher doses of PHY were needed to induce significant and dose-dependent increases in MYF when HY rats were the experimental subjects. LY rats showed salivation and muscle fasciculations with the higher doses used, suggesting that their sensitivity to other muscarinic autonomic or skeletal muscle effects was higher than in the HY subline. The regression lines for LY rats was calculated from the effects of 4 doses from 0.015 to 0.10 mg/kg, and for HY from 4 doses from 0.05 to 0.15 mg/kg ($r=0.88$ and 0.90, respectively). They are parallel ($t=0.933$). The estimated ED_{50}s are 0.032 mg/kg for LY rats and 0.085 mg/kg for HY animals. This shift represents a 2.6-fold increase in sensitivity to PHY in the former group.

2. Pilocarpine effects on spontaneous yawning behavior

Pilocarpine administration, within the range between 0.3 and 10 mg/kg, had a significant and dose-dependent yawn-inducing effect, in both HY (F19) and LY (F14–F15) sublines, with a latency as short as 5 to 6 min. These effects lasted for at least one hour. The highest dose used (10 mg/kg) produced noticeable side effects, intense salivation being the most remarkable. But no

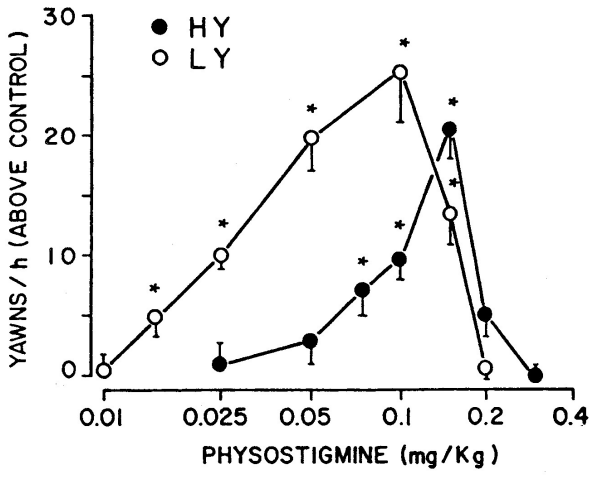

Fig. 1. PHY-induced yawning. Dose–effect curves. Ordinate: number of yawns elicited by PHY above spontaneous yawning frequency. Basal MYF: HY, 28 y/h; LY, 1.9 y/h; $n=12$ male rats per group. Statistics: KWT, $P<0.01$ for both curves; MWUT, $P<0.01$ or less when each group was compared with its control. Vertical bars represent S.E.M. Other details, see text.

differences in sensitivity towards pilocarpine were apparent between both sublines (Fig. 2).

Dopaminergic-induced yawning

1. APO effects

Fig. 3 compares APO-induced yawning in HY (F22–23) and LY (F20–21) sublines. Both dose–response curves are bell-shaped and similar to those obtained in other rat strains[3]. The regression lines, calculated from the effects obtained with 4 doses (0.01–0.10 mg/kg) for LY animals and 5 doses (0.001–0.025 mg/kg) for HY rats ($r = 0{,}597$ and $r = 0.627$, respectively), are parallel ($t = 0.00$). The ED_{50}s estimated from them by interpolation are: LY = 0.016 mg/kg; HY = 0.0025 mg/kg. The shift in sensitivity is 6.4-fold. With high doses of APO, above 0.3 mg/kg in HY rats, and 0.4 mg/kg upwards in LY rats, yawning was completely inhibited, confirming previous observations by several authors[3,14,15,22]

2. (−)3PPP effects

This drug has been described as a DA autoreceptor agonist with some antagonistic activity on D_2 postsynaptic receptors[9]. When its influence on yawning is explored in LY (F18–19) and HY (F22) animals, dose–response curves quite similar in shape are obtained (Fig. 4). There is a parallel shift to the left in the HY dose-response curve ($t = 1.08$), that suggests a slight increase in sensitivity (1.4-fold). HY animals treated with 10 and 25 mg/kg looked very sleepy; with 50 mg/kg

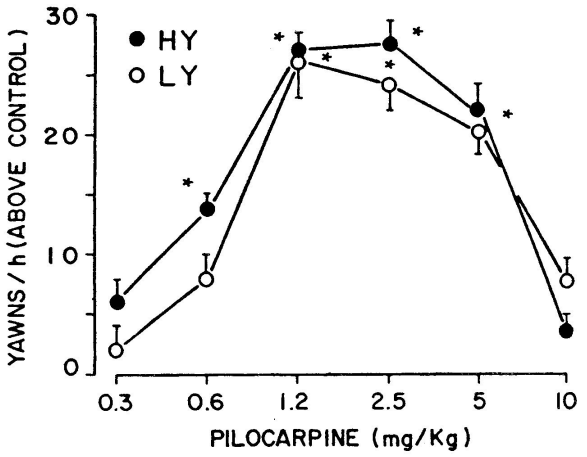

Fig. 2. Pilocarpine-induced yawning. Dose–effect curves. Ordinate: number of yawns induced by pilocarpine above spontaneous yawning frequency. Basal MYF: HY, 20.7 y/h; LY, 2.3 y/h. $n = 8$ male rats per group. Statistics: KWT, $P < 0.01$ for both curves; MWUT, $P < 0.01$ or less when each group was compared with its control. vertical bars represent S.E.M.

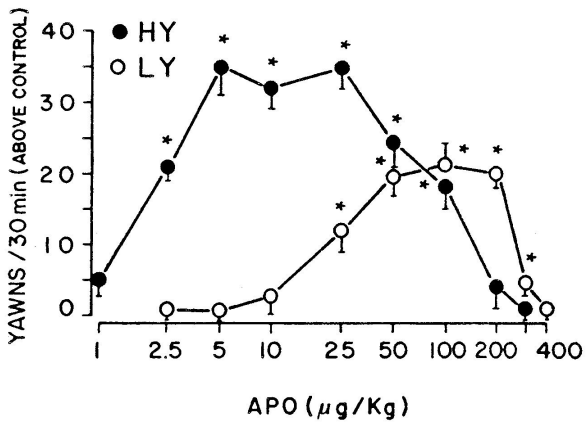

Fig. 3. APO-induced yawning. Dose–effect curves. Ordinate: number of yawns elicited by APO above spontaneous yawning frequency. Basal MYF: HY, 12,6 y/30 min; LY, 0.6 y/30 min. $n = 8$–10 male rats per group. Statistics: KWT, $P < 0.001$ for both groups; MWUT, $P < 0.01$ or less when each group was compared with its control. Vertical bars represent the S.E.M.

LY and HY rats appeared hypotonic and were immobile most of the time. This might explain the abrupt fall in yawning activity observed in both sublines with high doses of (−)3PPP.

DISCUSSION

The results reported above show that two sublines of Sprague–Dawley rats which differ in their spontaneous yawning frequencies[23] also differ in their responses to drugs with well known yawn-inducer activities.

It is known that the concentrations and turnover rates of several neurotransmitters, the activities of their synthesizing or degradative enzymes and their receptor densities in the brain show genetic variations[2,11,17,26,27,28]. Durkin et al.[5] have suggested that some changes in regional neurotransmitter activities in the brain might be due to primary differences in their underlying genetic mechanisms, whereas other might be secondary to imbalances in neurotransmitter interactions. Strain differences in behavioral expression or in sensitivity to drugs may be one major consequence of these changes.

Elicitation of yawning is mainly the result of an interaction, somewhere in the brain, between inhibitory dopaminergic and excitatory cholinergic influences on the built-in motor program for yawning[10,24,29]. Yawning induced by low doses of apomorphine or other DA agonists has most generally been interpreted as the result of their selective action on low-threshold DA autoreceptors regulating impulse discharge, synthesis and

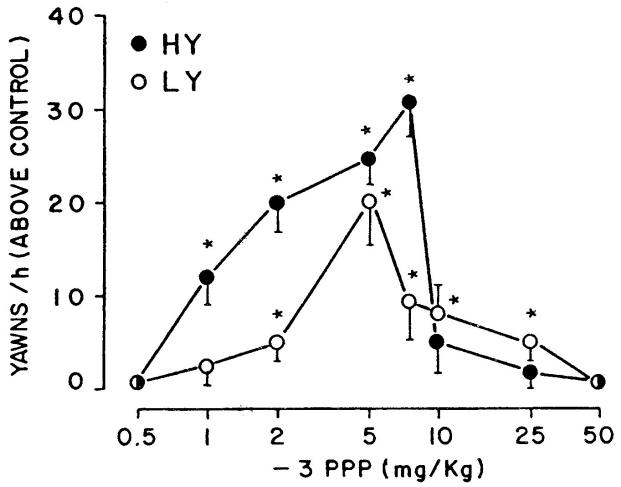

Fig. 4. 3PPP induced yawning. Dose–effect curves. Ordinate: number of yawns induced by this drug above spontaneous yawning frequency. Basal MYF: HY, 18.4 y/h; LY, 1.9 y/h. $n = 8–10$ male rats per group. Statistics: KWT, $P < 0.01$ for both curves; MWUT, $P < 0.03$ or less when each group was compared with its control. vertical bars represent the S.E.M.

release of the neurotransmitter[4,6,8,14,15,22,29,30]. Other authors have postulated that this effect could be due to postsynaptic excitatory DA_2 receptors[16,18]. On the other hand, higher doses of DA agonists decrease yawning by acting on high threshold postsynaptic DA yawn-inhibitory receptors[3,10,14,15,22]. In our previous paper[23] we had advanced the idea that HY rats may have an increase in cholinergic tone, understood as a direct and general effect, intrinsic to the cholinergic system as a whole, or as an indirect and more particular phenomenon, consecutive to a decrease in tonic DA inhibitory activity, and therefore restricted only to cholinergic pathways subject to dopaminergic restraining control. In the case of a primary difference affecting the cholinergic system as a whole, different sensitivities to cholinergic drugs, with no changes or smaller ones in the response to dopaminergic drugs, may be present when comparing HY and LY rats. The alternative hypothesis, that the primary genetic influences modifying yawning behavior in these two sublines are exerted on dopaminergic pathways may lead to differences in sensitivity both to dopaminergic drugs and secondarily to cholinergic drugs.

The results obtained with APO and (–)3PPP (Figs. 3 and 4), point to the latter alternative. Both drugs have been demonstrated to act on dopamine receptors and have been used to study the role of DA pathways on yawning and other behavioral patterns[3]. In HY rats the yawning dose–response curves traced with these dopaminergic agents are shifted to the left in relation to those in LY animals, indicating higher DA sensitivity in the former group. This suggests that the primary genetic difference between HY and LY rats is a decrease in the synthesis and/or release of dopamine in the former subline when compared with the latter. If this interpretation were correct, postsynaptic cholinergic neurones, partially disinhibited, would increase their activity, releasing more ACh and thus increasing MYF. The chronic increase in ACh might lead, as a secondary effect, to a diminution in sensitivity of muscarinic cholinoceptive receptors related to the yawning central pattern generator. We have tested this possibility by the use of pilocarpine, a well known muscarinic agonist and yawn-inducer[21]. We expected a diminution in pilocarpine-induced yawning in HY rats but our above described results do not agree with this prediction: no differences in pilocarpine-induced yawning responses are evident between HY and LY rats (see Fig. 2). The high turnover of ACh-esterase in the CNS could explain that although more ACh may be released in HY rats the steady average concentration of the neurotransmitter in synaptic space would not increase enough to determine changes in postsynaptic receptors' sensitivity. At this point it seems interesting to comment that Overstreet[17] has recently shown differences in muscarinic responses between Flinders-sensitive (FSL) and Flinders-resistant (FRL) lines of rats, differences that do not necessarily correlate with changes in receptors[17]. Nevertheless other results reported here show significant differences between the two sublines (HY and LY) in regard to physostigmine-induced yawning. Higher doses of this drug (a competitive inhibitor of AChE) are needed to induce yawning in HY rats. It is a well known fact that competitive enzyme inhibitors are less effective when their natural enzyme substrate is increased. The lower yawn-inducing effect of physostigmine in HY rats could be understood as due to a higher basal concentration of ACh as compared with LY animals.

In summary, the whole set of our results with yawn-inducing dopaminergic and cholinergic drugs suggest that the primary genetic difference between the HY and LY Sprague–Dawley sublines seems to affect the activity of dopaminergic inhibitory pathways that normally restrain yawning frequency.

ACKNOWLEDGEMENTS

This research was partially supported by CECyT-Puebla (Grant 0691-501), and by a generous gift of (–)3PPP from ASTRA, Sodertalje, Sweden. We deeply appreciate the help given to us by Alejandro Moyaho in the final editing of the manuscript.

REFERENCES

1 Anías, J., Holmgren, B., Urbá-Holmgren, R. and Eguibar, J.R., Circadian variation of yawning behavior. *Acta Neurobiol. Exp.*, 44 (1984) 179–186.

2 Buchner, E., Genes expressed in the adult brain of Drosophila. The effects of their mutations on behavior: a survey of transmitter- and second messenger-related genes, *J. Neurogenetics*, 7 (1991) 153–192.

3 Dourish, C.T. and Cooper, S.J., Neural basis of drug-induced yawning. In S.J. Cooper and C.T. Dourish (Eds.), *Neurobiology of Stereotyped Behaviour*, Oxford Clarendon Press, 1990, pp. 91–116.

4 Dourish, C.T., Herbert, E.N. and Iversen, S.D., Blockade of apomorphine-induced yawning in rats by the selective dopamine autoreceptor antagonist (+)-AJ 76, *Br. J. Pharmacol.*, 95 (1988) 498P.

5 Durkin, T.P., Hasseim-Zadeh, H., Mandel, P., Kempf, J. and Ebel, A., Genotypic variation in the dopaminergic inhibitory control of striatal and hippocampal cholinergic activity in mice, *Pharmacol. Biochem. Behav.*, 19 (1983) 63–70.

6 Ferrari, F., Sexual excitement and stretching and yawning induced by B-HT 920, *Pharmacol. Res. Commun.*, 17 (1985) 557–564.

7 Ferrari, W., Gessa, G.L. and Vargiu, L., Behavioral effects induced by intracisternally injected ACTH and MSH, *Ann. N.Y. Acad. Sci.*, 104 (1963) 330–345.

8 Gower, A.J., Berendsen, H.H.E., Princen, M.M. and Broekkamp, C.L.E., The yawning-penile erection syndrome as a model for putative dopamine autoreceptor activity, *Eur. J. Pharmacol.*, 103 (1984) 81–90.

9 Hjorth, S., On the mode of action of 3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine, 3-PPP, and its enantiomers, *Acta Physiol. Scand., Suppl.*, 517 (1983) 1–50.

10 Holmgren, B. and Urbá-Holmgren, R., Interaction of cholinergic and dopaminergic influences on yawning behaviour, *Acta Neurobiol. Exp.*, 40 (1980) 633–642.

11 Krehbiel, D., Bartel, B., Dirks, M. and Wiens, W., Behavior and brain neurotransmitters: correlations in different strains of mice, *Behav. Neural Biol.*, 46 (1986) 30–45.

12 Matsumoto, S., Yamada, K., Nagashima, M., Domae, M. Shirakawa, K. and Furukawa, T., Occurrence of yawning and decrease in prolactin levels via stimulation of dopamine-D_2-receptors after administration of SND919 in rats, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 340 (1989) 21–25.

13 Matsumoto, S., Yamada, K., Nagashima, M., Matsuo, M., Shirakawa, K. and Furukawa, T., Potentiation by serotonergic inhibition of yawning induced by dopamine receptors agonists in rats, *Pharmacol. Biochem. Behav.*, 32 (1989) 815–818.

14 Mogilnicka, E. and Klimek, V., Drugs affecting dopamine neurons and yawning behavior, *Pharmacol. Biochem. Behav.*, 7 (1977) 303–305.

15 Mogilnicka, E. Boissard, C.G. and Delini-Stula, A., Effects of apomorphine, TL-99 and 3-PPP on yawning in rats, *Neuropharmacology*, 23 (1984) 19–22.

16 Morelli, M., Longoni, R., Spina, L. and Di Chiara, G., Antagonism of apomorphine-induced yawning by SCH23390: evidence against the autoreceptor hypothesis, *Psychopharmacology*, 89 (1986) 259–260.

17 Overstreet, D.H., Commentary. A behavioral, psycho-pharmacological, and neurochemical update on the Flinders Sensitive Line rat, a potential genetic animal model of depression, *Behav. Genet.*, 21 (1991) 67–74.

18 Serra, G., Collu, M. and Gessa, G.L., Dopamine receptors mediating yawning: are they autoreceptors?, *Eur. J. Pharmacol.*, 120 (1986) 187–192.

19 Siegel, S., *Nonparametric statistics for the Behavioral Sciences*, Mac Graw-Hill, New York, 1956.

20 Tallarida, R.J. and Murray, R.B., *Manual of Pharmacologic calculations with computer Programs*, Springer, New York, 1981.

21 Urbá-Holmgren, R., González, R.M. and Holmgren, B., Is yawning a cholinergic response?, *Nature*, 267 (1977) 261–262.

22 Urbá-Holmgren, R., Holmgren, B. and Anías, J., Pre- and post-synaptic dopaminergic receptors involved in apomorphine-induced yawning, *Acta Neurobiol. Exp.*, 42 (1982) 115–125.

23 Urbá-Holmgren, R., Trucios, N., Holmgren, B., Eguíbar, R.R., Gabito, A., Cruz, G. and Santos, A., Genotypic dependency of spontaneous yawning frequency in the rat, *Behav. Brain Res.*, 40 (1990) 29–35.

24 Ushijima, H., Mizuki, Y. and Yamada, M., Multifocal sites of action involved in dopaminergic-cholinergic neuronal interactions in yawning, *Psychopharmacology*, 95 (1988) 34–37.

25 Ushijima, I., Mizuki, Y., Ukita, T., Kaneyuki, H., Inano, S. and Yamada, M., Behavioral effects of dilazep on cholinergic, dopaminergic and purinergic systems in the rat, *Pharmacol. Biochem. Behav.*, 43 (1992) 673–676.

26 Vadasz, C., Sziraki, I., Murthy, L.R. and Lajtha, A., Genetic determination of striatal tyrosine hydroxylase activity in mice, *Neurochem. Res.*, 11 (1986) 1139–1149.

27 Vadasz, C., Laszlovsky, I., De Simone, P.A. and Fleischer, A., Genetic aspects of dopamine receptor binding in the mouse and rat brain: an overview, *J. Neurochem.*, 59 (1992) 793–808.

28 Wallis, E., Overstreet, D.H. and Crocker, A.D., Selective breeding for increased cholinergic function: Increased serotonergic sensitivity, *Pharmacol. Biochem. Behav.*, 31 (1988) 345–350.

29 Yamada, K. and Furukawa, T., The yawning elicited by α-melanocyte-stimulating hormone involves serotonergic-dopaminergic-cholinergic neuron link in rats, *Naunyn Schmiedeberg's Arch. Pharmacol.*, 316 (1981) 155–160.

30 Zarrindast, M.R. and Poursoltan, M., Interactions of drugs acting on central dopamine receptors and cholino-receptors on yawning responses in the rat induced by apomorphine, bromocriptine or physostigmine, *Br. J. Pharmacol.*, 96 (1989) 843–848.