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INTERACTION OF CHOLINERGIC AND DOPAMINERGIC INFLUENCES ON YAWNING BEHAVIOR

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Abstract. The possible interaction between cholinergic and dopaminergic influences in the induction of yawning behavior in the rat is explored resorting to several experimental approaches: comparison of the ontogeny of yawning behavior induced by physostigmine (0.15 mg/kg) and apomorphine (0.05 mg/kg); simultaneous injection of both drugs; "crossed blocking" experiments, in which the action of the cholinomimetic agent is examined after injection of spiroperidol (0.05 mg/kg) and that of apomorphine after scopolamine (0.25 mg/kg). While physostigmine-elicited yawning is highest in early postnatal days and tends to decline from the 7th day onwards, reaching its lowest level around 3 wk, yawning induced by apomorphine begins around the 9th day and increases thereafter to a plateau that is reached in the third week. No synergism on yawning behavior is observed by simultaneous injection of optimal or suboptimal doses of physostigmine and apomorphine. Scopolamine blocks apomorphine-induced yawning; spiroperidol blocks apomorphine- but potentiates physostigmine-induced yawning, both in 15-day-old and young adult rats. Two 5-HT uptake blockers, citalopram (10–20 mg/kg) and fluoxetin (10–20 mg/kg) potentiate physostigmine- but not apomorphine-elicited yawning. On the basis of these results a tentative model of "in series" organization of dopaminergic and cholinergic influences on yawning behavior is proposed.

INTRODUCTION

Two different neurotransmitters, acetylcholine (ACh) and dopamine (DA), have been recently postulated among the neurohumoral me-

chanisms underlying the act of yawning. One group of investigators (11, 21), working on physostigmine- and pilocarpine-induced yawning in infant rats, has stressed the role of central muscarinic cholinergic synapses, because the yawning effect induced by both cholinomimetic drugs is blocked by scopolamine. On the other hand, Mogilnicka and Klimek (17) have described that low doses of systemically injected DA agonists in adult rats produce recurrent episodes of yawning, responses which are completely inhibited by the DA antagonist spiperone. Basically similar observations have been later reported by Di Chiara et al (6).

As physostigmine-induced yawning, both in infant and in 45-day-old rats, is strongly potentiated by previous administration of Lu 10–171 (citalopram), a potent and selective serotonin uptake blocking drug, a suggestion has also been advanced that serotonin (5–HT) may exert a modulating effect on yawning behavior (23).

Some questions immediately arise when these results are considered. Are the ontogenetic curves of yawning induced by cholinergic or dopaminergic drugs similar or different? May cholinergic and dopaminergic pathways somehow interact in the induction of yawning? Does serotonin also modulate dopaminergically-elicited yawning?

The experiments presented in this paper try to give an answer to these questions in the hope that a unified hypothesis regarding the neurohumoral basis of yawning may eventually emerge. This seems to be a relevant problem, since it has been shown that several other conditions or drugs are able to induce yawning: intraventricular infusions or intracerebral injections of adrenocorticotrophic hormone (ACTH) or fragments of ACTH (1, 8, 14), spreading depression of the neocortex (13) and hippocampus (14), and administration of naloxone (3).

MATERIAL AND METHODS

Experiments have been performed in both infant and young adult albino rats of a Wistar strain, carefully controlled regarding litter size, age and weight (11). When working with infant rats (less than 15 days old) both male and females were used indistinctly, the pups from each litter being equally distributed, at random, between experimental and control groups. In experiments with 15 day old rats or older animals only males were used, because it has been shown that young and adult female rats yawn significantly less than males when injected with cholinomimetic drugs (21).

Behavioral observations were restricted to the counting of yawns

during 30 min after the injection of the yawn-inducing drugs. For this purpose the animals were placed singly (adult rats) or in groups of two (infant rats) in transparent glass cylinders (diam., 18.5 cm; height, 9 cm), the floor being covered with a sheet of filter paper and the top with a transparent plastic plate. Experiments were run with four observers who ignored the particular drug or combination of drugs with which each animal had been injected and, as a rule, had no more than four animals to watch. All experiments were carried out in the morning hours (8.00–10.30).

Freshly prepared solutions of the following drugs were used: physostigmine (BDH Chemical Ltd.), apomorphine HCl (Sandoz), Lu 10–171.HBr or citalopram (H. Lundbeck and Co.), Lilly 110140. HCl or fluoxetin (Lilly Research Lab.), metergoline (Farmitalia), spiroperidol (Janssen Pharmaceutica) and scopolamine. HBr (Medexport). All drug doses are expressed in mg/kg. The drugs were dissolved in saline (NaCl 0.9%) so that the total volume to be injected i.p. was always equivalent to 0.01 ml/g body weight (infant rats) or less than 1 ml in adult rats. Spiroperidol was dissolved (2 mg/ml) in 100 mM tartaric acid and properly diluted to reach the desired concentration. Particular combinations or sequences of drug injections will be described in the following section. Controls received saline, and all animals were injected only once. Statistical procedures involved two standard nonparametric tests: the Kruskal Wallis Test for variance, and the Mann–Whitney U Test for comparison of groups (18).

RESULTS

Ontogenetic course of apomorphine-induced yawning. In this experiment, the idea was to compare the yawning-inducing effects of standard doses of apomorphine and physostigmine in animals ranging from 7 to 90 days old. For apomorphine 0.05 mg/kg doses was selected after a preliminary study of the dose-effect relationships of its yawn-inducing action in adult rats (90-day-old) (Fig. 1). The standard dose of physostigmine (0.15 mg/kg) was selected on the basis of previous experience (21) both with infant and adult rats.

Figure 2 shows that while in earlier postnatal days the effect of apomorphine is practically nil, its yawning-eliciting action grows with age, to reach an average of 5 yawns/30 min in 3 week old rats. Confirming previous observations (11), physostigmine-induced yawning is shown to decline from high levels in the early postnatal period to its lowest level in one month old rats. Spontaneous yawning at different ages is also shown in Fig. 2. From the 30th to the 90th day, both spontaneous and drug-induced yawning increase slightly.

Interaction of cholinergic and dopaminergic mechanisms in yawning. Three different experiments were performed in order to disclose if some interaction of cholinergic and dopaminergic influences might underly yawning behavior. First of all, the possibility of some synergism between apomorphine and physostigmine in the induction of yawning in young adult rats was explored. No summation of effects was observed when these two drugs were injected simultaneously, neither with doses optimal for a maximal yawning effect (apomorphine 0.05 mg/kg, physostigmine 0.15 mg/kg), nor with lower doses of one or the other drug.

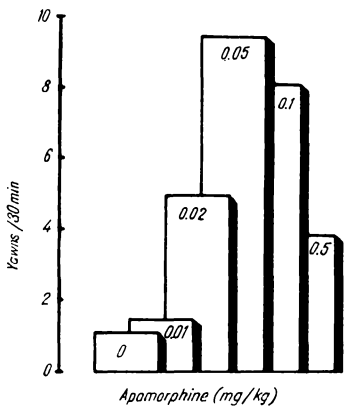

Fig. 1. Dose-effect relationships for apomorphine-induced yawning in adult male rats. Ordinates: number of yawns in 30 min. Drug doses are indicated in each column as mg/kg bodyweight. $N = 10$ animals per group. The quantitative differences in the yawning responses are significant (Kruskal–Wallis Test, $P < 0.05$). Differences between apomorphine injected animals and controls are: 0.01 mg/kg, NS; other doses $P < 0.01$ or less (Mann–Whitney U Test).

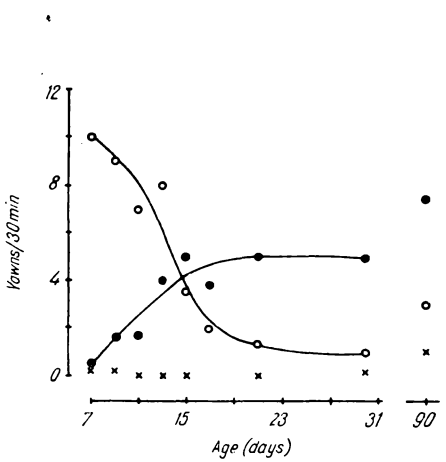

Fig. 2. Ontogeny of apomorphine- and physostigmine-induced yawning. Symbols: filled circles apomorphine 0.05 mg/kg; open circles physostigmine 0.15 mg/kg; crosses, controls, saline. $N = 12$ to 14 rats for each age. The quantitative differences in the responses at different ages with each of the drugs studied are significant (Kruskal–Wallis Test, $P < 0.05$). Drug effects statistically different from the controls at the following levels: apomorphine 7 days, NS; 9 and 11 days, $P < 0.02$; 13 d onward, $P < 0.001$. Physostigmine from 7 to 15 days $P < 0.001$; 17 days, $P < 0.01$; from 21 days onward, NS (Mann–Whitney U Test).

More suggestive evidence of interaction between cholinergic and dopaminergic synapses in the induction of yawning was obtained by "crossed blocking" experiments, in which the effect of an agonist upon

one of these two synaptic systems was explored in the presence of a specific antagonist of the other. Figure 3 illustrates the effect of blocking muscarinic cholinergic postsynaptic receptors with scopolamine (0.25 mg/kg) on yawning elicited by apomorphine (0.05 mg/kg). Yawning is almost entirely suppressed, as it had previously been demonstrated for physostigmine-induced yawning (22).

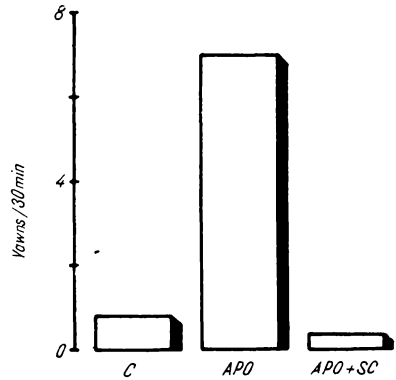

Fig. 3. Blocking effect of scopolamine on apomorphine-induced yawning. $N = 12$ to 14 3 month old rats (males) per group. Drug doses: apomorphine (APO), 0.05 mg/kg; scopolamine (Sc), 0.25 mg/kg. Controls (C) injected with saline. Differences between apomorphine injected animals versus the other two groups statistically significant, $P < 0.001$ (Mann–Whitney U Test).

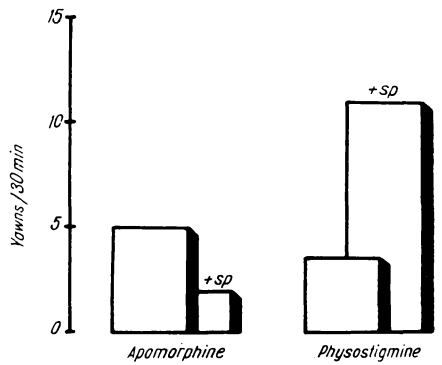

Fig. 4. Differential effect of spiroperidol on apomorphine- and physostigmine-induced yawning. $N = 16$ 15-day-old male rats. Drug doses: apomorphine, 0.05 mg/kg; physostigmine, 0.15 mg/kg, spiroperidol (Sp) 0.05 mg/kg. Differences are significant as follows: Apo. versus Apo. + Sp, $P < 0.01$; phys. vs.phys. + Sp, $P < 0.001$ (Mann–Whitney U Test).

The alternative experiment, i.e. testing the induction of yawning by physostigmine in the presence of a specific DA receptor blocking drug was also performed. For this purpose 15-day-old rats were used, because at this particular age no quantitative differences exist between the yawn-inducing effects of apomorphine and physostigmine (see Fig. 2). Spiroperidol (0.05 mg/kg) was injected i.p. one hour before the test with apomorphine or physostigmine, in standard doses. As may be observed in Fig. 4, while the antidopaminergic drug significantly blocked apomorphine-elicited yawning, a threefold potentiation of yawning behavior induced by physostigmine was obtained. Similar results have also been obtained in 3-month-old rats.

Serotonin effects on apomorphine-induced yawning. The question whether some serotonergic influences may be exerted upon dopaminergically-induced yawning, as has been suggested for the same behavior evoked by physostigmine (23), was explored in adult rats (3-month-old) with the aid of two serotonin uptake blocking drugs, citalopram and fluoxetin, and a serotonin receptor antagonist, metergoline. Figure 5 clearly presents that, neither citalopram nor fluoxetin exert any facilitating effects on apomorphine-induced yawning as they do on the same behavior elicited by physostigmine, when appropriate doses of the serotonin uptake blockers are used. Nevertheless, apomorphine-elicited yawning is subject to slight, but significant depression by metergoline (7.5 mg/kg) (Mann–Whitney U Test, $P < 0.05$).

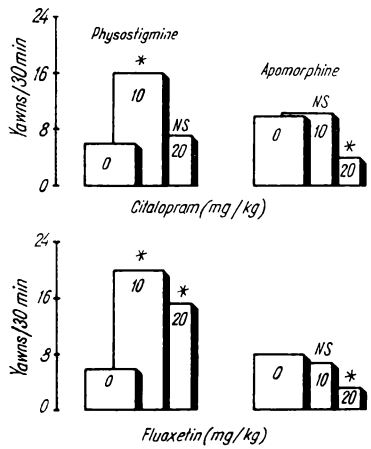

Fig. 5. Dose-response graphs for the effects of 5-HT uptake blockers on physostigmine- and apomorphine-elicited yawning. Ordinates: number of yawns during 30 min observation. $N = 12$ to 16 3-month-old male rats in each group. Drug doses: apomorphine, 0.05 mg/kg; physostigmine, 0.15 mg/kg; citalopram and fluoxetin doses are indicated in each column. Asterisks, indicate significance difference at the level of $P < 0.05$ or less; NS, non significant (Mann–Whitney U Test). The reduction in apomorphine-induced yawning with 20 mg/kg of both 5-HT uptake blockers was due to the rats falling asleep.

DISCUSSION

After Mogilnicka and Klimek's demonstration (17) that yawning may be elicited in adult rats by the administration of dopaminergic drugs, our previous suggestion (21) that yawning might be a cholinergic response needed re-examination. Cowan (5) had also pointed out that other factors, aparat from cholinergic mechanisms, could induce yawning. He recalled that i.m. administration of dimethyltryptamine causes yawning in rhesus monkeys. DPI, (3,4 dihydroxyphenylamino)-2-imidazoline, which has been described to have specific and potent agonistic activity on DA inhibitory receptors (20), also elicits yawning in infant rats (22).

In the present experiments, using apomorphine in the same low dose range as Mogilnicka and Klimek (17), we have confirmed their and Di Chiara et al. (6) results, showing that this drug definitely eli-

cits yawning in adult male albino rats. We have also traced the ontogenetic course of this effect. Apomorphine does not induce yawning in 7-day-old rats, does it only slightly before the eleventh day, but quite distinctly by the 15th day, when the effect practically reaches the same level observable until the end of the first month. A 50% higher average effect is obtained in adult rats (3-month-old). Increase with age of other behavioral effects of DA agonists has recently been reported (19). The ontogenetic evolution of apomorphine-elicited yawning thus follows an absolutely different curve than the one which illustrates the same behavior evoked by physostigmine or pilocarpine (11). Cholinomimetically-induced yawning is highest during the first seven postnatal days, falling thereafter to a level which is not significantly different from spontaneous yawning on the 21st day. The different maturation course of cholinergic- and dopaminergic-induced yawning responses naturally suggest different underlying mechanisms. Nevertheless, the fact that both cholinomimetic- (21) and apomorphine-elicited yawning are blocked by low doses of scopolamine (Fig. 3), points to the decisive importance of some muscarinic cholinergic synaptic links in the neuroanatomical circuits subserving yawning. How could one envisage the coupling of dopaminergic and cholinergic synapses in order that yawning might be elicitable both by dopamino- or cholinomimetic agents and subject to block by scopolamine whatever the eliciting drug? One possible explanation is offered by the tentative and simple model presented in Fig. 6, in which DA and ACh synapses appear organized "in series", the latter exerting an excitatory influence on the central pattern generator of yawning behavior, and thus being able to trigger the response. The early maturation of yawning, both spontaneous and cholinomimetically-induced (11) justifies the suggestion that the central pattern generator "yawning center" and its cholinergic trigger may have a quite caudal location in the brain stem. This is in keeping with the observation of yawning in anencephalic humans (15), with the opinion of the early reviewers of yawning behavior (2, 10) and the most recent description of yawning in a completely tetraplegic patient suffering from a transecting glioma of the pons (9).

Figure 6 also suggests that the cholinergic yawn-triggering neurons are under the control of later maturing inhibitory dopaminergic neurons. In rats, their gradual restraining influence on yawning behavior may be illustrated by the declining tendency of cholinomimetic yawning from around the 7th postnatal day onwards, which corresponds roughly to the reciprocal evolution of apomorphine-elicited yawning. In agreement with previous authors (6, 17) we interpret apomorphine-induced yawning as the result of activation of low threshold presynap-

tic inhibitory DA receptors, "autoreceptors" of Carlsson (4), by low doses of apomorphine, thus liberating the cholinergic neurons triggering yawning from a tonic dopaminergic restraining influence. Higher doses of apomorphine or other DA agonists, by directly stimulating high threshold postsynaptic DA inhibitory receptors on the cholinergic

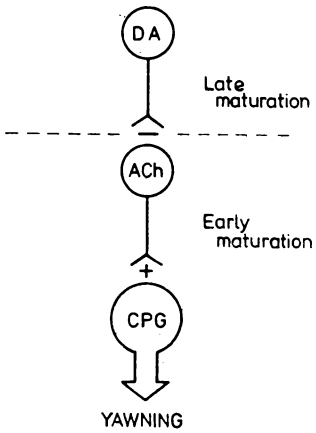

Fig. 6. Hypothetical organization of cholinergic and dopaminergic influences on yawning. CPG, central pattern generator of yawning. For explanation see text.

neurons, would exert an inhibitory effect on yawning. The dose-effect of apomorphine-induced yawning (Fig. 1) demonstrates the very narrow dose range of this effect, which begins to decline already with 0.20 mg/kg. This seems to be the reason why authors studying stereotyped activity with higher doses of apomorphine (7, 12, 16) failed to observe its yawning-eliciting action. If this interpretation is correct it could be expected that if excessive apomorphine is injected (for an optimal yawn-eliciting effect), and the concentration of the drug falls, as a result of metabolic degradation, late yawning activity might appear. We have confirmed this expectation in a group of 5 young adult rats injected with apomorphine (1 mg/kg). In that case significant yawning appeared only in the second half of a 60 min observation period while in rats injected with 0.05 mg/kg yawning began already 5 min after injection and had practically disappeared after half an hour (unpublished observations).

The "crossed blocking" experiment (Fig. 4), in which physostigmine-induced yawning is increased after blocking DA receptors with spiroperidol, while the same behavior elicited by apomorphine is decreased, provides further evidence in support of the idea that an important dopaminergic tonic inhibitory influence on yawning is present around the 15th postnatal day in the rat. The potentiating effect of spiroperidol on physostigmine-induced yawning is not significant in rats 7 to 11 days old, in concordance with the relative immaturity of the dopaminergic

influences on yawning, as illustrated by the ontogenetic course of apomorphine-elicited yawning (Fig. 2).

An important serotonergic facilitatory effect on physostigmine-induced yawning has been previously described (23). If DA and ACh synapses influencing yawning were organized as suggested in Fig. 6, it would be reasonable to expect that serotonin uptake inhibitors should have a similar effect on apomorphine-elicited yawning as they have on that behavior evoked by physostigmine (23). Our experiments testing the action of citalopram and fluoxetin on yawning induced by apomorphine failed to demonstrate such effect (Fig. 5). This is contradictory to the demonstration that a significant depression of apomorphine-elicited yawning is obtainable with metergoline and, therefore, difficult to explain. Thus, the idea that serotonergic pathways may exert a general tonic facilitatory or modulatory influence on yawning (23), although appealing, should perhaps require further substantiation.

The exploration of the effect of drugs modifying dopaminergic and cholinergic synaptic transmission upon yawning induced by other methods (1, 3, 8, 13, 14) may help in testing the more general validity of the tentative model of organization of the synaptic links regulating and triggering yawning behavior presented in this work (Fig. 6).

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