PRE- AND POST-SYNAPTIC DOPAMINERGIC RECEPTORS INVOLVED IN APOMORPHINE-INDUCED YAWNING

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Key words: yawning, dopaminergic receptors, apomorphine

Abstract. The temporal course of yawning behavior elicited by increasing doses of apomorphine (APO), from 0.01 to 10 mg/kg, was studied experimentally in adult albino rats. In the higher dose range a great prolongation of drug induced yawning latency is observed. This result is explainable by postulating differences in sensitivity of two sets of dopaminergic (DA) receptors: low threshold presynaptic DA receptors which, when activated, disinhibit yawning, and high threshold postsynaptic DA receptors inhibiting yawning. Apomorphine in high doses can entirely suppress physostigmine-elicited yawning.

INTRODUCTION

Several authors have described yawning behavior in the rat after the administration of apomorphine in low doses (5, 11, 16). Higher doses tend to produce other stereotyped movements, as sniffing, licking or gnawing (3, 6, 8–10). This difference in the behavioral patterns that are induced has been interpreted as due to activation of different sets of

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dopaminergic receptors. Low doses of apomorphine or other dopamine (DA) agonists, seem to stimulate preferentially low threshold inhibitory receptors localized on the soma, the dendrites and the presynaptic membrane of the axon terminals of dopaminergic neurons. Quite a strong evidence suggests that this set of "autoreceptors" (1) exerts an inhibitory influence both on the synthesis and liberation of DA, and on the frequency of nerve impulse discharges in dopaminergic neurons (1, 2, 4, 12, 13). High doses of direct DA agonists would also activate the higher threshold postsynaptic DA receptors, thus inducing (by excitation or inhibition) the physiological or pharmacological effects mediated by the postsynaptic neurons. In the case of yawning behavior it has been proposed that DA neurons exert a tonic inhibition of cholinergic neurons exciting this motor behavior (5, 16). Thus, if the postsynaptic dopaminergic effect is inhibitory, presynaptic DA receptor activation would disinhibit yawning.

In this work we present complementary results to those published recently by our group in this journal (5). They were basically obtained by a detailed study of the temporal course of apomorphine's yawning effect, and are interpreted in the framework of Melzacka et al. (9, 10) studies on the pharmacokinetics of apomorphine (APO) and the correlation of its concentration in different structures of the brain with particular stereotyped behavioral patterns.

**MATERIALS AND METHODS**

Experiments were performed on 216 male, 3–4 months old albino rats of a Wistar strain, which were used only once. The observation of yawning was made as previously described (5). For the comparison of the dose–effect curves obtained after s.c. and i.p. administration of APO, yawns were counted during one hour after the injection. The study of the temporal course of APO-induced yawning involved longer periods of observation (up to 4 h) and the clocking of each yawn on a 1 min time basis.

Two drugs were used: apomorphine. HCl (Chimimport, Bulgaria) and physostigmine (BDH, England). The solutions were freshly prepared in distilled water, and properly diluted in saline (0.9% NaCl) to a total volume of 0.20 ml/100 g body weight to be injected. In the case of APO solutions, ascorbic acid was added to the distilled water in a proportion of 0.20 mg/ml, to hinder oxidation of the drug. Drug doses are expressed in mg/kg of the respective base. Statistical procedures will be mentioned with the results.
RESULTS

Dose-effect relations. In our previous work with APO (5) we systematically used the i.p. route for the administration of the drug, but on several occasions we observed rats in which yawning was not elicitable even with optimal doses of APO (0.05 to 0.10 mg/kg). As the liver seems to be the most important site for apomorphine metabolic degradation (3, 14) i.p. injections do not seem recommendable for tracing a dose-effect curve, specially in the lower dose range. We have, therefore, made a comparison between the dose-effect relations in APO-induced yawning when using the s.c. route (lumbar region) with those obtained by i.p. injections (Fig. 1). Some slight differences are observable. Results with 0.01 mg/kg, which are not significantly different from the controls injected with saline by the i.p. route, reach a level of significance \( P < 0.05 \) by s.c. injections. While 28% of the rats injected i.p. with 0.05 mg/kg did not exhibit yawning, this percentage was reduced to only 8% when using the s.c. route. With 0.10 mg/kg yawning occurrence reached 100%, whatever the injection route utilized.

![Dose-effect graph of APO-elicited yawning](image-url)
Yawning chronograms. The temporal pattern of distribution of individual yawns in drug-induced yawning is quite variable from animal to animal. Nevertheless, in the case of APO-elicited yawning some interesting tendencies emerge when the responses to increasing doses of the drug are compared. A graphic illustration of individual yawns plotted against time (yawning chronograms) is shown in Fig. 2. It includes representative cases for several doses. As may be seen, the optimal responses are obtained with 0.05 mg/kg. Two different patterns may appear with 1 mg/kg. The most typical is characterized by a prolongation of the latency to the first yawn observed after the moment of injection, to 20 min or more. But in a couple of cases, after an early burst of one to three yawns, response frequency falls, and only scattered yawns may be observed during the following half an hour. A more stable response ensues, which covers the following 20 min, to disappear about one hour after the injection of apomorphine. The injection of still higher doses, from 5 to 10 mg/kg, introduces noteworthy prolongations of the latency to the first yawn, from 90 min to more than two hours, as illustrated in Fig. 2.

In Fig. 3 we have plotted the latencies to the first yawn in all the experiments performed, against the doses of APO, on a log/log scale.
Fig. 3. Correlation between yawning latencies and APO doses. Each point represents the response of one adult male rat. Calculated regression line: $r = 0.838$; $P < 0.01$.

Fig. 4. Correlation between yawning $T_{50}$ and APO doses. Each point represents the value obtained in one adult male rat. Calculated regression line: $r = 0.954$; $P < 0.001$. For explanations, see text.
The calculated regression line has a correlation coefficient of 0.921 ($P < 0.001$). In order to examine quantitatively the whole yawning response in function of time and APO dose, the concept of $T_{50}$ may be introduced. We define it as the time, after APO injection, in which 50% of the total number of yawns, clocked during one hour of observation after the first yawn, has been reached. A graph of the experimental data analysed in this way is shown in Fig. 4. The correlation coefficient of the log/log regression line is 0.954 ($P < 0.001$).

Interaction of apomorphine- and physostigmine-elicited yawning in function of time. Conceiving that the prolongation of latency in apomorphine-induced yawning behavior elicited with higher drug doses might

Fig. 5. Interaction of APO- and physostigmine-induced yawning. Upper part, yawning chronograms. Lower graph: dose-effect relations. Columns represent the mean number of yawns during one hour after physostigmine (Phys), APO or both drugs administered simultaneously in the doses indicated within each column. Each group consisted of 12 adult male rats. Difference between Phys + APO injected rats and APO group are significant as follows: $P < 0.05$; $P < 0.01$ (Wilcoxon and Wilcox multiple comparison test (15).
be an expression of postsynaptic dopaminergic inhibition of cholinergic neurons triggering the response, such a hypothesis could possibly be tested by simultaneous administration of apomorphine and physostigmine, the latter drug in its optimal yawn-inducing dose (0.15 mg/kg). This experiment is illustrated in Fig. 5. The normal response to physostigmine, injected alone, is represented by the uppermost yawning chronogram. When optimal doses of both physostigmine and apomorphine are injected simultaneously, no significant change is observed compared to the result obtained with APO alone. The response to physostigmine disappears completely when this drug is combined with higher doses of apomorphine (0.5 to 5 mg/kg). As the effects of physostigmine disappear, around 40–45 min after injection, the later yawning responses observed must correspond to those evoked by apomorphine alone. In the lower part of Fig. 5 a conventional dose-effect graph is presented, which includes the average values from groups of twelve animals, injected with solutions containing the standard optimal dose of physostigmine (0.15 mg/kg) and increasing doses of apomorphine, yawns being counted during an observation period of 1 h. On comparing this representation of the data with their respective chronograms it becomes clear that the fall in response to the higher doses of apomorphine is in great measure a result of the prolongation of yawning latency, because yawns produced after 60 min are evidently not counted.

DISCUSSION

Basically similar results have been reported by the research groups that have studied apomorphine-elicited yawning behavior (5, 11, 16). This DA agonist, in low doses not producing hypermotility or increased exploratory activity, nor typical stereotyped motor patterns as sniffing, licking or gnawing, tends to evoke hypomotility and recurrent episodes of yawning and penile erections. Interpretation of these observations is also quite coincidental as the yawning effect is most probably due to activation of low threshold presynaptic DA self-inhibitory receptors. Nevertheless, the possibility that "a special kind of postsynaptic DA receptors ... different from DA receptors responsible for motor stimulation, stereotypy and arousal for being more sensitive to the action of this transmitter" (2) might be responsible for the hypnotic effect of low doses of apomorphine, and perhaps also for yawning, erection and ejaculation, has not been excluded.

Some quantitative differences in the results described by different authors deserve some comments. Yamada and Furukawa's (16) apomorphine-induced dose-effect curve obtained with i.p. injections shows its
highest average yawning rate of about 4 yawns/hour with doses in the 0.25 to 1 mg/kg range, with an occurrence between 62.5 to 87.5%. Our results indicate an average yawning rate of approximately 10–13 yawns/hour for s.c. administration of apomorphine (0.05–0.1 mg/kg) and around 8–9 yawns/h when the drug was injected i.p. At a dose of 0.1 mg/kg yawning occurrence reaches 100% in our experiments, with the use of both the s.c. and the i.p. route of administration. The difference between the Japanese author's (16) and our results can thus be only partially explained by differences in the route of drug administration. We therefore tend to think more of the possibility of genetic differences, both in APO sensitivity and yawning responsiveness between their and our strains of rats. By inbreeding rats with high incidence of spontaneous yawning we have obtained in the second generation males that yawn spontaneously at an average rate of 5 yawns/h when they reached the age of 45 days (unpublished results), a rate which is higher than Yamada and Furu-kawa's average level for APO-induced yawning (16).

In Melzacka et al. (9) studies on the distribution of apomorphine in the rat's brain, it was demonstrated that APO is rapidly absorbed from the bloodstream by the brain tissue, and that its disposal from the latter has a half-time of around 15 min. Because of differences in the level of APO in specific brain areas, coinciding with the time of appearance of particular stereotyped symptoms (sniffing, licking or gnawing), the authors suggest that each symptom appears when the APO concentration reaches a critical level in a given area of the brain. We suppose that this group did not observe yawning in their rats because their experiments were of too short duration for the high APO dose range (2.5 to 20 mg/kg) within which they worked, range in which yawning would only appear, according to our experiments, after a latency of at least 1–3 h (see Fig. 3). In a later work made in the same laboratory (10) a standard dose of 1 mg/kg was used to study the correlation between the above mentioned behavioral effects and cerebral pharmacokinetics of APO. They observed the rat's behavior for at least 10 min to calculate the incidence of sniffing, not less than 15 min for licking, and at least 25 min for gnawing, because, according to their experience “if these symptoms do not appear within the specified periods of time, they do not appear at all”. Such a conclusion might be valid for these typical high dose apomorphine-induced stereotyped motor patterns, but evidently not from yawning, as our results with 5 to 10 mg/kg clearly indicate.

Accepting Melzacka et al. idea (9) that a particular behavior appears when APO concentration reaches a critical level in a certain area in the brain, we may add the suggestion that, in the case of yawning, an upper level exists above which yawning is blocked. We propose that the
lower critical level for yawning is represented by the threshold of those presynaptic DA receptors which, activated by APO, disinhibit yawning, and that the upper level might correspond to the threshold of postsynaptic DA receptors inhibiting the cholinergic neurons that excite yawning. Thus, yawning would be elicited while APO concentration in the CNS is maintained between these two critical levels. The proposition is schematized in Fig. 6. The upper part of the figure is based on the general temporal course of APO concentrations in the brain after three increasing doses of the drug, as described by Melzacka et al. (9). The lower part of the figure depicts the corresponding yawning chronograms. Yawning is evoked when APO concentration in the brain surpasses line 1 and is inhibited when the drug's concentration exceeds line 2. Case a is self-evident. When a higher dose, b, is injected, yawning would be induced both during the early period of increasing concentration of APO in the brain and, again, after a pause, when APO's concentration falls beneath the upper yawn-inhibitory threshold. With even higher doses,
c, the concentration of apomorphine in the brain passes abruptly across the yawn-eliciting range (between 1 and 2) into the inhibitory one, so that a yawn effect is only observable after a long latent period, during the late falling phase, when APO concentration is declining from critical level 2 to 1.

REFERENCES


Accepted 30 November 1981