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GABAergic Modulation of Yawning Behavior

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DOGER, E., R. URBÁ-HOLMGREN, J. R. EGUIBAR AND B. HOLMGREN. *GABAergic modulation of yawning behavior.* PHARMACOL BIOCHEM BEHAV **34**(2) 237–240, 1989.—The hypothetical modulation by GABAergic neurons of yawning behavior in the rat was explored with GABA-active drugs. Gamma-acetylenic-GABA, a specific inhibitor of GABA-T, increases yawning frequency when injected at a dose of 7 mg/kg. Baclofen, a $GABA_B$ agonist (3 mg/kg), inhibits yawning completely; GABA antagonists, bicuculline and picrotoxin, at subconvulsant doses, also decrease yawning. All drugs were injected intraperitoneally with the exception of apomorphine, which was injected subcutaneously. It is suggested that $GABA_B$ receptors play a role in yawning behavior by modulating ACh release, and that $GABA_A$ receptors may modify yawning frequency by modulating inhibitory influences on ACh neurons.

Yawning GABA $GABA_A$ receptors $GABA_B$ receptors

YAWNING is a discrete innate motor pattern widely represented in the behavioral repertoire among vertebrates (5, 17, 18). It occurs spontaneously at variable frequencies (2,4), and is subject to a complex set of neurotransmitter and hormonal influences (6, 12, 13, 24, 27–29, 35, 43). Yawning can be induced by cholinomimetic drugs such as physostigmine and pilocarpine, and is inhibited by scopolamine (35, 39, 40). It may also be evoked by low doses of apomorphine (APO), and other dopamine (DA) receptor agonists (− 3PPP and bromocriptine) (20, 29, 32, 37, 38, 43). With higher doses of APO, which also act upon postsynaptic receptors, spontaneous and physostigmine-induced yawning are inhibited (20, 32, 37, 38). In spite of the fact that other substances, like the peptide hormones ACTH (12,13), α-MSH (12), prolactin (24) and oxytocin (3), are yawning inducers, several authors agree in assigning a crucial role in the regulation of yawning to the DA-acetylcholine (ACh) interaction (15, 37, 42).

Links between ACh and DA neurons have been properly described in the septo-hippocampal pathway and the striatum (1, 16, 34). It has been described that stimulation and lesion of these structures affect yawning frequency (10, 21, 25, 33, 43).

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the principal efferent systems related to the nigro-striatal and the ventral tegmental-nucleus accumbens dopaminergic systems (26,31). Also, it is well known that GABAergic neurons regulate dopaminergic and cholinergic activities in these structures (30, 31, 41). Therefore, it seemed important to explore the role of the GABA system in the regulation of yawning behavior. Two different types of GABA receptors have been described, $GABA_A$ and $GABA_B$. The former are sensitive to muscimol and THIP, and are antagonized by bicuculline. Receptors of this kind are mostly situated postsynaptically, and are coupled to chloride channels. The other type of GABA receptors, $GABA_B$, are not sensitive to bicuculline, their most specific agonist is baclofen; they are mostly presynaptic and are involved in the modulation of ACh release (8, 11, 22, 23).

The experiments here reported have been performed with GABAmimetic drugs and GABA antagonists. The use of two sublines of rats with high and low spontaneous yawning frequency has allowed us to study the effect of these GABA-active drugs, both on spontaneous and drug-induced yawning.

METHOD

Animals

Male Sprague-Dawley rats, 2–3 months old, 200–300 g in weight, from two sublines selectively bred to establish high (HY) and low (LY) yawning frequency were used. They were bred in our animal house under standard conditions: light-dark cycle 12:12 hr, lights on from 0700 to 1900; food (Purina chow) and water were supplied ad lib. The animals were weaned at the age of 30 days and maintained in groups of four in collective Plexiglas cages.

Drugs

The drugs used in the experiments were the following: gamma-acetylenic-GABA (GAG) (Merrel Labs, France); bicuculline methiodide (Sigma, USA); picrotoxin (Sigma, USA); tetrahydroisoxipiridonol HCl (THIP) (Research Biochem. Inc., USA); α-p-chlorophenyl-GABA (baclofen) (Ciba Geigy, Basel, Switzerland); physostigmine sulphate (Sigma, USA); apomorphine HCl (APO) (Chimimport, Bulgary); and 3-(3-hydroxyphenyl)-N-n-propyl piperidine) HCl (− 3PPP) (Astra, Sweden). Bicuculline was dissolved in 0.1 N HCl and diluted with saline. The other drugs were directly dissolved in saline. Solutions were freshly prepared before the experiments. A standard injection volume of 0.2 ml/100 g body weight was used. APO was injected subcutaneously (SC). Other drugs were administered intraperitoneally (IP). Doses are expressed as mg (base)/kg body weight. GAG was injected eight

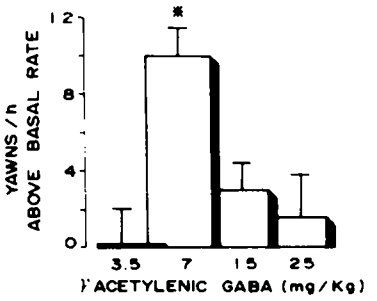

FIG. 1. Effect of GAG on spontaneous yawning frequency. Ordinate: number of yawns above basal rate per hour: mean basal yawning rate (MBYR) = 19.4 yawns/hr. Abscissa: GAG doses. n = 10 HY male rats per dose (Kruskal-Wallis test, $p<0.05$, Mann-Whitney U-test, $*p<0.05$). Vertical bars indicate standard error (SE).

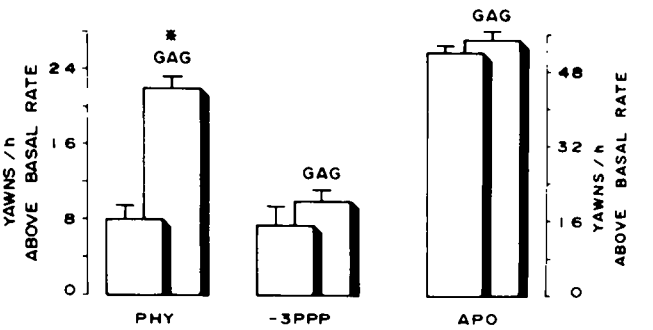

FIG. 2. Effect of GAG on pharmacological-induced yawning. Ordinate: number of yawns above basal rate per hour; PHY = physostigmine, 0.15 mg/kg, IP, MBYR = 25.4 yawns/hr; − 3PPP, 10 mg/kg, IP, MBYR = 19.3 yawns/hr; APO = apomorphine 0.05 mg/kg, SC MBYR = 22.1 yawns/hr; GAG 7 mg/kg, IP. n = 10 HY male rats per group (Mann-Whitney U-test, two-tailed, $*p<0.05$). PHY and − 3PPP results are referred to the left ordinate. For each combination of drugs, two groups of animals were compared: one of them was pretreated with GAG injected 8 hr before, the other with saline, and both received the indicated yawn-inducing drug. The three yawn-inducing drugs increase basal yawning rates significantly (Mann-Whitney U-test, $p<0.05$ or less). Vertical bars indicate SE.

hours before the observation period, − 3PPP 15 minutes before, and all the other drugs immediately before yawn monitoring began. Animals were used only once. Statistical procedures will be mentioned with the results.

Behavioral Observations

Observations were performed with each animal placed in a transparent glass cylinder (diameter 19 cm, height 10 cm), the floor of which was covered with a sheet of clean filter paper, and the top with a Plexiglas plate, leaving a segment 1 cm wide open for ventilation. The observation periods were between 1700–1800 hr. Yawns were monitored by two observers sitting on opposite sides of the table on which the animals were placed. Usually eight rats were observed simultaneously.

RESULTS

GAG, a specific inhibitor of GABA-transaminase (the catabolic enzyme of GABA), exerts GABAmimetic activity with a long latency. Its maximal effects on yawning are observed 8 hours after administration (9). Therefore, in these experiments, the rats were injected 8 hr before the behavioral observations.

The dose-response curve of GAG on spontaneous yawning was plotted with four doses (Fig. 1). A significant increase in yawning was obtained only with 7 mg/kg; with higher doses (15 and 25 mg/kg) the animals appeared very sleepy and hypodynamic. In order to study the effects of the drug on pharmacologically-induced yawning we used the following experimental design: 10 HY male rats, pretreated with GAG (7 mg/kg, 8 hr before), were injected with the yawning inducer and observed during 1 hr. Figure 2 shows the results when physostigmine (0.15 mg/kg IP), APO (0.05 mg/kg, SC) and − 3PPP (10 mg/kg IP, 15 min before observation) were used as inducers. The increase in yawning frequency, observed in GAG-physostigmine-injected rats, was the only significant result obtained ($p<0.05$, Mann-Whitney U-test).

In other experiments, we tested THIP, a $GABA_A$ agonist, and baclofen, a $GABA_B$ agonist, in both HY and LY rat sublines. Systemic administration of THIP, at 0.33, 1.0, 3.0 and 6.0 mg/kg, does not produce any change in yawning frequency (Kruskal-Wallis, $p>0.05$). On the other hand, baclofen produces a clear inhibition of spontaneous yawning behavior (Fig. 3) (Kruskal-Wallis, $p<0.05$; Mann-Whitney U-test, $p<0.05$ or less). Baclofen also inhibits physostigmine-induced yawning (Fig. 3).

The effects of GABA antagonists, bicuculline and picrotoxin, were also tested in HY male rats. Both drugs, at subconvulsant doses, reduce spontaneous yawning frequency. With bicuculline the highest effect was obtained with 3 mg/kg, while 1 mg/kg picrotoxin suppressed yawning completely (Fig. 4) (Kruskal-Wallis, $p<0.05$; Mann-Whitney U-test, $p<0.05$ or less).

DISCUSSION

In order to evaluate the possible role of the GABA system in the regulation of yawning we studied the effects of different GABA-active drugs upon this behavior.

The decrease in spontaneous yawning frequency observed with baclofen ($GABA_B$ agonist) suggests that GABA neurons play a role in yawning regulation. This decrease may be caused by a lower central cholinergic tone, due to a presynaptic GABAergic modulatory action on cholinergic terminals. This sort of modula-

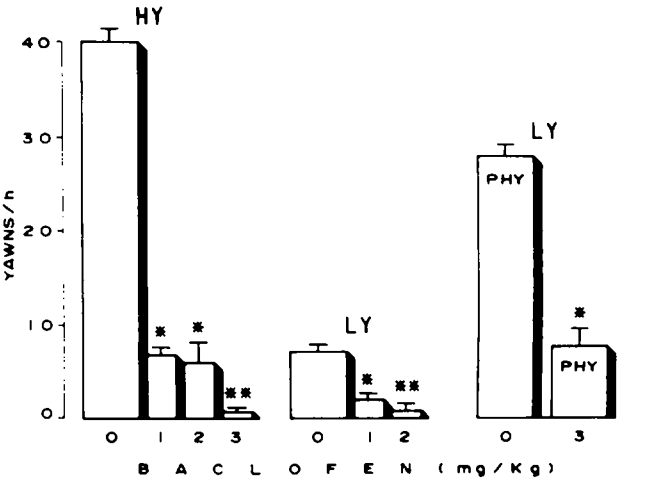

FIG. 3. Effect of baclofen on spontaneous and physostigmine-induced yawning. Ordinate: number of yawns observed during 1 hr. Abscissa: baclofen doses (mg/kg) IP. PHY = physostigmine 0.15 mg/kg, IP, n = 12 male rats per dose. LY and HY dose-response curves: Kruskal-Wallis test, $p<0.05$; Mann-Whitney U-test, * and $**p<0.05$, and $p<0.02$ when compared with the control. Vertical bars indicate SE.

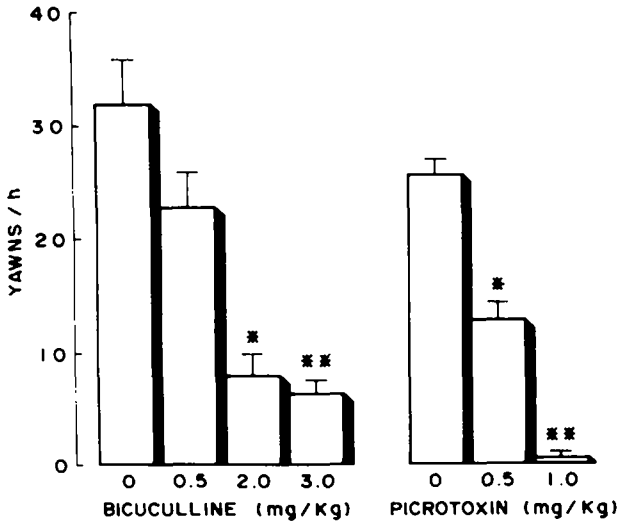

FIG. 4. Dose-response curves of bicuculline and picrotoxin on yawning behavior. Ordinate: numbers of yawns observed during 1 hr. Abscissa: drug doses in mg/kg; n = 10 male rats per dose. Bicuculline and picrotoxin dose-response curves: Kruskal-Wallis, $p<0.05$; Mann-Whitney U-test, $*p<0.05$, $**p<0.02$. Vertical bars indicate SE.

tion has been described in both peripheral and central nervous systems (7,14).

Seeking further evidence to support the idea that baclofen acts by modulating ACh release, we tested its effect upon physostigmine-induced yawning. Since physostigmine effects, as an indirect cholinergic agonist, depend on ACh being released, we should expect that baclofen would decrease physostigmine-induced yawning frequency. Our results (Fig. 3) agree with this assumption.

Administration of THIP ($GABA_A$ agonist) does not change yawning frequency. At a first glance, this might mean that only $GABA_B$ receptors participate in the regulation of this behavior. Nevertheless, the increase of spontaneous and physostigmine-induced yawning obtained with GAG (Figs. 1 and 2), suggests that GABA may also inhibit an inhibitory pathway acting upon yawning trigger neurons (Fig. 5). The reduced yawning frequency observed with bicuculline ($GABA_A$ antagonist) (Fig. 4) also supports this statement. On the other hand, the GAG dose-response curve (Fig. 1)—with only one effective dose—might be understood as due to GABA effects on two sets of GABA receptors with different sensitivities and opposite actions on yawning behavior.

Summarizing, our results suggest that GABA neurons play a role in yawning regulation at two sites: 1) controlling ACh release at cholinergic terminals, through $GABA_B$ receptors and 2) modulating the activity of dopaminergic yawning-inhibitory influences through $GABA_A$ receptors.

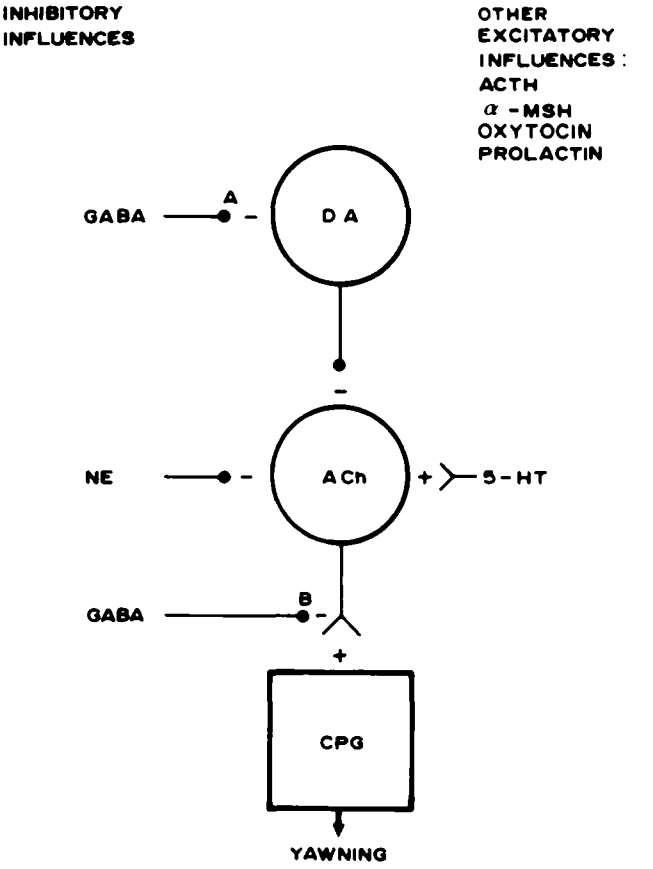

FIG. 5. Hypothetical model which illustrates neurotransmitter and hormonal influences on yawning behavior, and suggested sites of action of GABA. DA = dopaminergic neuron; ACh = cholinergic neuron; GABA; 5-HT = serotonin; NE = noradrenaline; ACTH = adrenocorticotrophic hormone; α-MSH = melanocyte stimulating hormone; CPG = central pattern generator of yawning behavior. (−) Inhibitory and (+) excitatory influences. References to these different inhibitory and excitatory influences may be found in the Introduction.

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