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BRIEF COMMUNICATION

Blockade of both Pilocarpine and Amphetamine-Induced Head-Shaking with Dopamine Receptor Antagonists

BJORN HOLMGREN, RUTH URBÁ-HOLMGREN AND BOCAR DEM

Centro Nacional de Investigaciones Científicas
Apartado 6990, La Habana, Cuba

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HOLMGREN, B., R. URBÁ-HOLMGREN AND B. DEM. *Blockade of both pilocarpine- and amphetamine-induced head-shaking with dopamine receptor antagonists.* PHARMAC. BIOCHEM. BEHAV. 8(6) 767–769, 1978. – The range of central dopaminergic mechanisms involved in both d-amphetamine- and pilocarpine-induced head-shaking was studied in 7–9 days old rats by means of two DA antagonists: pimozide and spiroperidol. Both blocking agents exert their effects on H–S following dose-response curves which are similar, whatever the drug used to evoke head-shaking. A complete blocking effect on H–S is reached with pimozide at a dose of 2 mg kg^{-1}; with spiroperidol at 0.1 mg kg^{-1} (for pilocarpine-induced H–S) and at 0.2 mg kg^{-1} (for H–S evoked by d-amphetamine). These results, together with those previously reported, suggest that dopaminergic and cholinergic facilitatory influences on H–S seem to be organized in series.

d-Amphetamine Pilocarpine Pimozide Spiroperidol Dopaminergic-cholinergic interactions
Infant rats Head-shaking

INFANT albino rats tend to shake their heads spontaneously [8]. This tendency is markedly increased by d-amphetamine and, to a less degree, by apomorphine [9] and pilocarpine [7]. On the other hand, d-amphetamine induced head-shaking (H–S) is inhibited by chlorpromazine, haloperidol, phenoxybenzamine [9] and also by scopolamine [7]. Altogether these results indicate that catecholaminergic and cholinergic influences on H–S are strongly concurrent and not organized in a balance between antagonistic CA and ACh activities, as is currently accepted for different functions of the brain [1, 3, 4, 5, 11, 12, 13].

The facts that scopolamine blocks d-amphetamine induced H–S completely, and that pilocarpine potentiates it in the order of 400% [7] stress the importance of the cholinergic facilitatory influences, and suggest that they might be in series with the dopaminergic mechanisms.

With the aim of exploring the range of DA contributions to H–S we have performed experiments in which the effects of two potent DA antagonists on D-amphetamine and pilocarpine induced H–S are compared.

METHOD

The animals were offspring (7 to 9 day-old) of Wistar rats raised in our Animal House. The results are based on 80 litters, which were reduced to eight pups on the day following delivery.

Animals from each litter were randomly assigned to the experimental and control groups and tested only once. Their bodyweights were within ± SEM [8].

The following substances were used: d-amphetamine sulphate (Rhône Poulenc), pilocarpine (Boehringer), pimozide (Janssen) and spiroperidol (Janssen). Spiroperidol was dissolved (2 mg ml^{-1}) in 2 mM tartaric acid; pimozide (2.5 mg ml^{-1}) in 100 mM tartaric acid. Both solutions were properly diluted with distilled water to reach the desired concentrations. Other drugs were dissolved in physiological saline (NaCl 0.9%).

Drug solutions were freshly prepared and injected IP at the standard volume of 0.01 ml g^{-1} bodyweight. Control animals received only the drug vehicle. The doses given refer to the form indicated above.

Immediately after injection of the H–S inducer (amphetamine or pilocarpine) each pup was placed in a glass cylinder (18 cm dia.), the floor of which was covered with a piece of filter paper, and observed for 15 min. Pups which regularly shaked their heads during this time were injected with the antidopaminergic drugs or with the vehicles (controls).

Duration of the H–S episodes were measured with stop watches during the following 45 min. The blocking effect of pimozide and spiroperidol on H–S is expressed as the percent reduction in total H–S time at 60 min after injection of amphetamine or pilocarpine. The statistical procedures are described with the results.

RESULTS

Pimozide, in doses equal or higher than 2 mg kg^{-1} bodyweight, suppresses H–S induced either by d-amphetamine or by pilocarpine by five min after IP injection (Fig. 1). Lower doses affect H–S with a longer latency or in

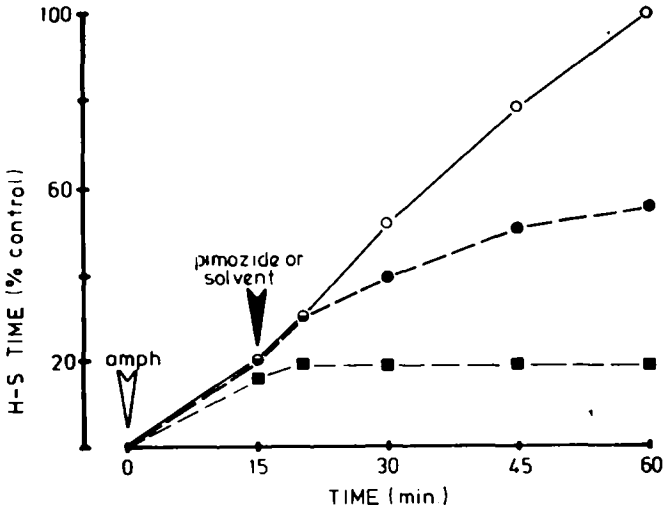

FIG. 1. *Effect of pimozide on d-amphetamine-induced H–S.* Abscissa: total mean H–S time; during 1 hr of observation. The arrows indicate the moments in which drugs were injected to 7–9 day-old rats. N=12 for each group. ○, control; ●, pimozide 0.25 mg kg^{-1}; ■, pimozide 2 mg kg^{-1}. Values after drug injection are significantly different from the controls as follows: pimozide 2 mg, 30 min, $p<0.05$; 45 min, $p<0.01$; and 60 min, $p<0.006$. Pimozide 0.025 mg kg^{-1}: non-significant at any time.

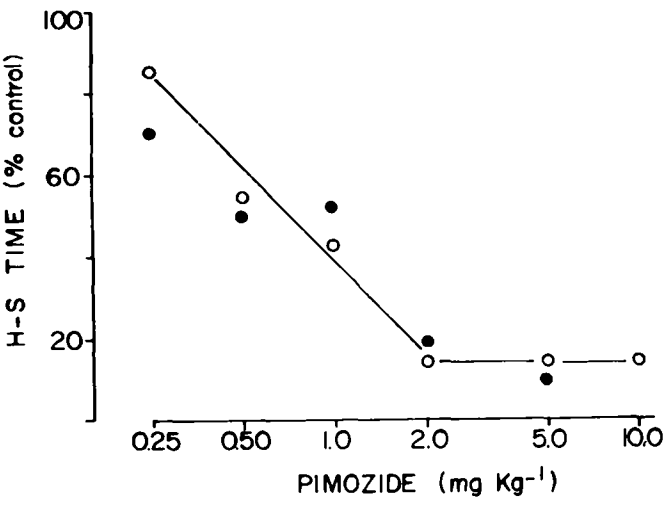

FIG. 2. *Dose-response curve for pimozide effect on d-amphetamine and pilocarpine-induced H–S.* Abscissa: Total mean H–S, during 45 min of observation after pimozide administration; ○, d-amphetamine- and ●, pilocarpine-induced H–S. Each point represents the mean value for 12–14 rats, 7–9 day-old. Line was drawn with d-amphetamine results. Regression on a semilogarithmic curve indicates proportionality for both sets of data between drug effects and doses in the range 0.25–2.0 mg kg^{-1} (amphetamine r: 0.80, $p<0.01$ and pilocarpine: r: 0.73, $p<0.01$). Other details in the text.

a more gradual fashion. The dose-response curve for the blocking effect of pimozide on d-amphetamine induced H–S is shown in Fig. 2, in which data corresponding to the same effect on H–S evoked by pilocarpine are also included. It is obvious that quite similar effects were obtained, independently from the H–S inducing drug.

After a variance analysis (Kruskal-Wallis, $p<0.01$ for both sets of data), Mann-Whitney U tests between controls and pimozide treated animals revealed that the only non-significant inhibition was obtained with 0.25 mg kg^{-1}; all other groups differ significantly from their respective controls at the level of $p<0.05$ for 0.50 and 1 mg kg^{-1}, and $p<0.001$ for 2 to 10 mg kg^{-1}. Figure 3 shows that spiroperidol also inhibits H–S evoked either by amphetamine or pilocarpine (Kruskal-Wallis, $p<0.01$ for both sets of data), the effects being statistically different when compared with the controls within the range of $p<0.02$ for 0.05 mg kg^{-1} to $p<0.001$ for 0.20 mg kg^{-1} (Mann-Whitney U test).

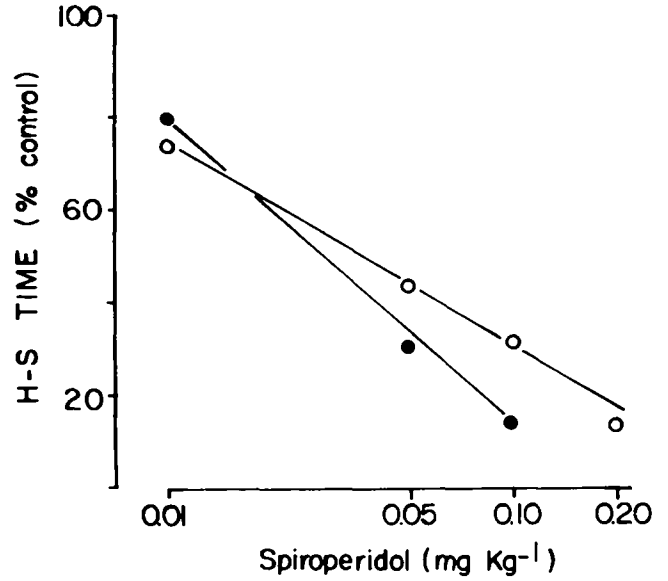

FIG. 3. *Dose-response curve for spiroperidol action on H–S induced by d-amphetamine or pilocarpine.* Abscissa: Total mean H–S time, during 45 min of observation after spiroperidol administration. ○, d-amphetamine- and ●, pilocarpine-induced H–S. Each point represents the mean value for 12–14 rats, 7–9 day-old. For both sets of data regression on a semilogarithmic curve indicates proportionality (d-amphetamine r: 0.74, $p<0.01$ and pilocarpine r: 0.78, $p<0.01$). Other details in the text.

DISCUSSION

The fact that pimozide, an antidopaminergic drug with a relatively high anticholinergic potency (ACh/DA = 0.87) [10], may completely block amphetamine and pilocarpine induced head-shaking is not surprising in consideration to the double blocking effects of the drug. But that spiroperidol, which has negligible anticholinergic activity (ACh/DA = 0.008), may also do so, is quite striking. This is strongly suggestive that in head-shaking induced by pilocarpine, a dopaminergic synaptic link is involved, just as cholinergic synaptic activity participates in d-amphetamine induced H–S. One might conceive the existence of a common neural subsystem, including a pacemaker or an oscillating neural circuit, and facilitatory dopaminergic, noradrenergic and cholinergic influences, as the structuro functional mechanism of head-shaking. Although not discarding the idea that supraspinal pacemakers might be involved in H–S as in other cholinergically induced tremors [2, 6, 14] we have formerly suggested that this motor

symptom might be due to the intrinsic tendency of the segmentary reflex arcs controlling head-rotatory neck muscles to oscillate at a frequency which is strongly age-dependent [8]. In experiments designed to compare the oscillatory frequency of H–S induced by d-amphetamine and by pilocarpine (B. Holmgren and R. Rodríguez, unpublished observations) we have observed that if due care is taken to compare animals of the same age and at the same body temperature, H–S frequencies are practically identical.

The facilitatory influences of dopaminergic and cholinergic pathways on H–S seem to be organized in series, because this motor symptom is entirely suppressed by scopolamine, pimozide or spiroperidol.

Different seems to be the case for noradrenergic influence, which might be operating in parallel with the dopaminergic and cholinergic ones, since only partial blockade of d-amphetamine induced H–S was observed with the adrenergic blocking drug phenoxybenzamine [9].

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