ALPHA-ADRENERGIC BLOCKING PROPERTIES OF DROPERIDOL ON ISOLATED BLOOD VESSELS OF THE DOG

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SUMMARY

Concentrations of droperidol which caused a shift to the right of the dose-response curve to noradrenaline in the pulmonary artery and the saphenous vein of the dog did not affect myogenic activation by K+; they did not inhibit spontaneous activity of portal-mesenteric veins. Droperidol inhibited the contractile response to nerve stimulation, but did not affect the evoked release of ³H-noradrenaline. These experiments indicate that the vasodilator properties of smaller doses of droperidol are a result of its ability to block alpha-adrenergic receptors.

In the intact organism, the neuroleptic drug droperidol has been shown to antagonize the pressor response to catecholamines. This effect of droperidol has been attributed to its ability to block alpha-adrenergic receptors (Janssen et al., 1963; Schaper, Jageneau and Bogaard, 1963; Yelnosky, Katz and Dietrich, 1964; Whitwam and Russell, 1971). However, in the isolated rabbit ear artery droperidol was reported to cause a non-specific inhibition of vasoconstrictor responses, and its alpha-adrenergic blocking properties have been questioned (Puddy, 1971). The present experiments were performed to gain further insight into the mechanism by which droperidol depresses vasomotor responses in isolated blood vessels.

MATERIAL AND METHODS

The experiments were performed on helical strips of saphenous veins and of pulmonary arteries, and on longitudinal strips of anterior mesenteric veins taken from dogs (15–25 kg) anaesthetized with pentobarbitone 30 mg/kg i.v. The strips were placed in a chamber filled with 50 ml of Krebs-Ringer solution (NaCl, 118.3 mmol; KCl, 4.7 mmol; MgSO₄, 1.2 mmol; KH₂PO₄, 1.2 mmol; CaCl₂, 2.5 mmol; NaHCO₃, 25 mmol; calcium EDTA, 0.026 mmol; glucose, 11.1 mmol). The solution was maintained at 37 °C and aerated with 95% oxygen in 5% carbon dioxide.

The preparations were placed at the optimal point of their length-tension relationship (Vanhoutte and Leusen, 1969), and were allowed to equilibrate for 90 min before the experiments commenced.

³H-noradrenaline efflux. Pulmonary artery strips were incubated for 4 h in Krebs-Ringer solution containing 7-³H-noradrenaline at 3 x 10⁻⁷ M (specific activity, 8.8 Ci/mmol, Amersham/Searle Corporation, Des Plaines, Ill.). The strips were rinsed in fresh solution, and mounted for isometric tension recording. Two platinum wires were placed parallel to the arteries. Both the vessel and the electrodes were perfused continuously (3 ml/min) by means of a roller pump. Droperidol or a solvent was added to the perfusate upstream from the pump. The perfusate was collected for direct estimation of the total radioactivity or for chromatographic analysis. ³H-noradrenaline was separated from non-catechols and from deaminated metabolites (3,4-dihydroxymandelic acid, and 3,4-dihydroxyphenyl-glycol) as described previously (Vanhoutte, Lorenz and Tyce, 1973; Muldoon, Verbeuren and Vanhoutte, 1976; Vanhoutte et al., 1976).

Drugs. The following drugs were used: droperidol, 1-noradrenaline bitartrate, phentolamine and propranolol. Droperidol was obtained by diluting with distilled water a solution of 1.3 x 10⁻³ M of the agent in 2.5 x 10⁻² M of the solvent (tartaric acid); preliminary experiments indicated that the used concentrations of tartaric acid did not affect reactivity of the vessels studied. When droperidol was given, the control strips, as described previously (Vanhoutte and Leusen, 1969). When drugs were added to the bath solution, they were contained in 0.1 ml. In certain experiments, the Krebs–Ringer solution in the bath was replaced with solution containing added KCl in equimolar replacement for NaCl.

The preparations were placed at the optimal point of their length–tension relationship (Vanhoutte and Leusen, 1969), and were allowed to equilibrate for 90 min before the experiments commenced.
preparations received the corresponding amount of solvent; if the control preparation was the same strip, the control response was obtained in the presence of the amount of tartaric acid needed to dissolve the highest concentration of droperidol used. The other drugs were dissolved in distilled water. All doses are expressed as final concentrations (molar) in the bath solution or in the perfusing fluid.

Analysis of data. For each group of preparations, the number of strips reported is also the number of dogs used. The data are expressed as means ± SEM. For statistical evaluation of the data, Student's t test for paired or unpaired observations was used. P values < 0.05 were considered significant.

RESULTS

Unstimulated preparations

Two tissues from six spontaneously active mesenteric veins were studied in parallel, and exposed to droperidol $1.3 \times 10^{-8}$ to $2.6 \times 10^{-5}$ M or solvent, respectively. Droperidol $2.6 \times 10^{-5}$ M depressed significantly the amplitude of the spontaneous contractions (from $2.52 \pm 1.18$ to $1.75 \pm 0.72$ g). More dilute concentrations of droperidol, and the solvent, did not have significant effects on rhythmic activity. In five pulmonary arteries and 11 saphenous veins, droperidol $1.3 \times 10^{-8}$ to $2.6 \times 10^{-8}$ M did not alter the baseline tension.

Stimulated preparations

Exogenous noradrenaline. Two tissues were prepared from each of five pulmonary arteries. Four cumulative dose–response curves to noradrenaline $5 \times 10^{-8}$ to $5 \times 10^{-4}$ M were obtained in Krebs–Ringer solution and in solutions containing solvent or droperidol $1.3 \times 10^{-7}$, $1.3 \times 10^{-6}$ and $1.3 \times 10^{-5}$ M, respectively. In the control tissues, there was a progressive increase in the maximal response to noradrenaline with time (fig. 1; left); droperidol caused a dose-dependent, parallel, and significant shift to the right of the dose–response curve (fig. 1; right).

In five saphenous veins dose–response curves to noradrenaline $10^{-8}$ to $10^{-4}$ M were compared in the absence and in the presence of droperidol (fig. 2). Droperidol $1.3 \times 10^{-8}$ and $1.3 \times 10^{-7}$ M caused a shift to the right of the dose–response curve to the catecholamine. In the presence of droperidol $1.3 \times 10^{-6}$ and $1.3 \times 10^{-5}$ M, the maximal response was obtained with noradrenaline $5 \times 10^{-5}$ M and was significantly depressed; noradrenaline $10^{-4}$ M caused a significant relaxation. In six further strips treated with propranolol $1.5 \times 10^{-5}$ M, droperidol $1.3 \times 10^{-6}$ M caused a parallel shift to the right of the response to lower concentrations of noradrenaline, but significantly depressed the maximal response to the catecholamine; the relaxation caused by noradrenaline $10^{-4}$ M was no longer seen (fig. 3).
DROPERIDOL AND VASCULAR SMOOTH MUSCLE

Fig. 2. Comparison of the response of dog saphenous vein strips to noradrenaline in Krebs-Ringer solution containing solvent (control) and increasing concentrations of droperidol. Data shown as means for five veins. Symbols are as used in figure 1.

Nerve stimulation. In six pulmonary arteries, droperidol $1.3 \times 10^{-7}$ M caused a marked depression of the frequency-response curve to electrical impulses (1–10 Hz); in the presence of droperidol $1.3 \times 10^{-5}$ M, the preparations no longer reacted to electrical stimulation (fig. 4; left). In six saphenous vein strips, droperidol $1.3 \times 10^{-7}$ and $1.3 \times 10^{-5}$ M caused a significant, dose-dependent, reduction of the response to all stimulation frequencies tested (fig. 4; right).

$\text{3H-noradrenaline efflux.}$ Two tissues from each of six pulmonary arteries were studied simultaneously for tension changes and $\text{3H-efflux.}$ Electrical stimulation (5 Hz) was imposed for 2 min; this caused an increase in tension, total radioactivity and efflux of $\text{3H-noradrenaline}$ and its metabolites (fig. 5). One strip then received solvent and the other droperidol $1.3 \times 10^{-6}$ M. The latter caused a significant increase

Fig. 3. Effect of droperidol $1.3 \times 10^{-6}$ M on response of dog saphenous vein to increasing concentrations of noradrenaline. Solvent and propranolol $1.5 \times 10^{-6}$ M are present throughout the experiment. Data shown as means for five strips. • = difference from control value is statistically significant.

Fig. 4. Effect of increasing concentrations of droperidol on response of dog pulmonary artery (left) and saphenous vein (right) to electrical stimulation at increasing frequencies. Data shown as means for six preparations in each group. Symbols are as in figure 1.
in efflux of 3,4-dihydroxyphenylglycol (from $15.4 \pm 3.5$ to $25.4 \pm 5.7 \times 10^3$ DPM/6 min). The contractile response to 5 Hz was significantly depressed in the preparations exposed to droperidol; however, the increase in efflux of tritiated compounds was not significantly different from that obtained in the control strips (fig. 5). The chromatographic analysis of the perfusate indicated that droperidol did not significantly affect the evoked release of $^3$H-noradrenaline and its metabolites.

**Potassium ions.** Two tissues were prepared from five saphenous veins; in these experiments all solutions contained phentolamine $10^{-5}$ M and propranolol $1.5 \times 10^{-5}$ M. In the control tissues the contractile response to increasing K$^+$ concentrations (30–70 mmol/litre) increased with time. There was no significant difference between the results obtained in the control tissues and those treated with droperidol $1.3 \times 10^{-7}$ and $3 \times 10^{-4}$ M (fig. 6).

**DISCUSSION**

The principal aim of this study was to determine whether or not droperidol has specific alpha-adrenergic blocking properties in isolated vascular smooth muscle. Some previous reports have suffered from the uncertainty that the solvents and preservatives used were responsible in part for the effects ascribed to droperidol (Greene, 1972). No preservatives were used in the present study, and a solvent was selected which does not affect basal tension or reactivity to noradrenaline; data obtained with droperidol were systematically compared with those obtained in the presence of solvent.

**Effects on smooth muscle cells.** In the pulmonary artery, droperidol causes a dose-related parallel shift to the right of the noradrenaline dose–response curve, although there is no evidence of a depression of the maximal response to the catecholamine. Thus, in this preparation, droperidol can be regarded as an alpha-adrenergic receptor blocking drug.

In the saphenous vein, droperidol $1.3 \times 10^{-8}$ M causes also a parallel shift of the dose–response curve to noradrenaline, indicative of alpha-adrenergic blocking activity. At higher concentrations, droperidol still shifts the response to lower concentrations of noradrenaline to the right in a parallel manner; however, the maximal response is depressed, and the highest concentration of noradrenaline causes relaxation. This relaxation must be a result of unmasking of the beta-adrenergic receptor stimulating properties of high concentrations of noradrenaline, since it is abolished by propranolol. In the presence of propranolol, the highest concentration of droperidol used in the present study still causes depression of the maximal response to noradrenaline; this suggests that in the saphenous vein, droperidol in high concentrations may act as a non-competitive inhibitor of the
response to the catecholamine. Since, for the lower concentrations of noradrenaline, the dose–response curve remains shifted in parallel, this non-competitive effect of high doses of droperidol probably is either not related to the alpha-adrenergic receptors or only involves part of the receptor population.

In the rabbit ear artery lower concentrations of droperidol depressed the response to increases in K⁺ concentration, which led to the conclusion that it is a non-specific vasodepressant (Puddy, 1971). However, in adrenergically innervated blood vessels, K⁺ causes contraction in part by releasing endogenous noradrenaline (Lorenz and Vanhoutte, 1975). Thus, relaxation observed during K⁺-evoked contractions may be a result of alpha-adrenergic inhibition. Direct evidence for this interpretation comes from the experiments in which saphenous veins were treated with phentolamine and propranolol to mask the adrenergic components of the K⁺-induced responses. In these conditions concentrations of droperidol, which cause a parallel shift of the response to noradrenaline, do not depress that to increases in the concentration of K⁺. In this regard droperidol differs from local anaesthetic agents, which in concentrations inhibiting noradrenaline-induced contractions of saphenous vein strips, depress their responses to K⁺ to the same extent (Muldoon, Verbeuren and Vanhoutte, 1976). Thus, in concentrations which possess alpha-adrenergolytic properties, droperidol does not cause myogenic depression. This conclusion is supported further by the data obtained from spontaneously active mesenteric veins in which droperidol causes depression of the rhythmic contractions only at concentrations greater than 10⁻⁶ M.

Effect on nerve endings. In the saphenous vein and in the pulmonary artery, the contractions caused by electric impulses are a result of release of endogenous catecholamines rather than to direct activation of the smooth muscle cells (Shepherd and Vanhoutte, 1975). Droperidol causes a significant depression of the response to electrical stimulation in the pulmonary artery and in the saphenous vein. This depression could be explained either by the alpha-adrenergic blocking properties of the drug, or by an inhibitory effect on the release of noradrenaline.

In the absence of nerve stimulation droperidol augments the efflux of deaminated metabolites in pulmonary arteries previously incubated with ³H-noradrenaline; this effect is similar to that seen with amide-linked local anaesthetics, which has been
attributed to an increased leakage of noradrenaline from the storage vesicles, making more transmitter available for deamination within the nerve (Muldoon, Verbeuren and Vanhoutte, 1976). However, unlike the local anesthetic agents, droperidol does not decrease the evoked release of \(^3\)H-noradrenaline in a concentration which significantly depresses the tensile response. This demonstrates that the inhibition of the response to nerve stimulation is a result of the alpha-receptor blocking action of droperidol on the smooth muscle cells.

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PROPRIETES DE BLOCAGE ADRENERGIQUE ALPHA DU DROPERIDOL SUR LES VAISSEAXES SANGUINS ISOLES DU CHIEN

RESUME

Les concentrations de droperidol qui provoquent une déviation vers la droite de la courbe dose/réaction à la noradrénaline dans l’artère pulmonaire et la veine saphène du chien n’ont pas affecté l’activation myogénique par K\(^+\); elles n’ont pas modéré l’activité spontanée des veines porte et mesenteriques. Le droperidol a modéré la réaction contractile à la stimulation nerveuse, mais il n’a pas affecté le dégagement évocé de noradrénaline \(^3\)H. Ces expériences indiquent que les propriétés vasodilatatrices de plus petites doses de droperidol sont le résultat de sa capacité de blocage des récepteurs adrénergiques alpha.

SUMARIO

Concentraciones de droperidol que provocaron una desviación a la derecha de la curva dosis-respuesta a la noradrenalina en la arteria pulmonar y la vena safena del perro no afectaron la activación miogénica por K\(^+\); no inhibieron la actividad espontánea de las venas portal-mesentéricas. Droperidol inhibió la respuesta contractil a la estimulación nerviosa, pero no afectó la liberación evocada de \(^3\)H-noradrenalina. Estos experimentos indican que las propiedades vasodilatadoras de dosis más pequeñas de droperidol son resultado de su facultad de bloquear a los receptores alfa-adrenérgicos.