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Interaction between clonidine and histamine on the guinea-pig isolated trachea

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In experiments on guinea-pig isolated tracheal spirals, clonidine, in concentrations of 10^{-6} to 3×10^{-4} M, had a contracting effect which was strongly inhibited by prazosin but not significantly modified by yohimbine. Moreover, clonidine (3×10^{-5} to 3×10^{-4} M) potentiated histamine-induced contractions; this latter effect was inhibited specifically by α_1-adrenoceptor antagonists (e.g. prazosin, AR-C 239) but unmodified by yohimbine, nicardipine or agents acting on the arachidonic acid cascade. It would therefore appear that clonidine in high concentrations contracts the guinea-pig trachea by stimulating α_1-adrenoceptors and that, contrary to what has been reported with other animal species, notably the dog, the guinea-pig trachea is devoid of α_2-adrenoceptors that mediate contractions.

α_1-Adrenoceptors of the tracheobronchial tree have been the subject of numerous binding and pharmacodynamic studies as have α_2-adrenoceptors located in the same tissues. Bronchospasm induced by acetylcholine, histamine or 5-hydroxytryptamine in the conscious guinea-pig is enhanced by clonidine in doses of 10 to 100 $\mu g\ kg^{-1}$, but it has no contracting effect on the guinea-pig isolated trachea, at concentrations of up to 10^{-5} M (Advenier et al 1983). In contrast, clonidine (10^{-7} to 10^{-6} M) produces strong contractions of the dog isolated trachea (Barnes et al 1983), which has been shown by binding studies to contain α_2-adrenoceptors. These effects of clonidine in the conscious guinea-pig and on the dog trachea are specifically inhibited by α_2-adrenoceptor antagonists such as yohimbine and piperoxane.

The purpose of the present study was to evaluate the effects of clonidine on the guinea-pig isolated trachea and to find out whether it was capable of contracting the respiratory smooth muscle in this animal as it does in the dog.

Methods

Tracheal spirals were obtained from male guinea-pigs (250–350 g) anaesthetized with urethane ($1{\cdot}25\ g\ kg^{-1}$ i.p.) and pretreated 18 h previously with reserpine $5\ mg\ kg^{-1}$, i.m. Spirals were equilibrated under an initial tension of 1·20 g in Krebs solution (NaCl 114, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25 and glucose 11·7 mM) maintained at 37 °C and gassed with 5% CO_2 in O_2. Tension was measured isometrically with a Gould strain gauge (ref. UC3) connected to a Bryans BS 2H recorder. The initial tension ensured that after a 1·25 h equilibration period the resting tension was between 0·4 and 0·8 g. Under these conditions responses to spasmogenic agonists were reproducible. The bathing solution contained cocaine (10^{-5} M) and propranolol (10^{-6} M) to reduce interference from uptake systems and β-adrenoceptor effects.

In all preparations, the tracheal spirals were first contracted to maximal tension with histamine $2{\cdot}2 \times 10^{-4}$ M.

In one series of experiments, cumulative clonidine concentration-response curves were established on tracheas kept under resting tone without pretreatment other than cocaine and propranolol or pretreated 15 min before with prazosin or yohimbine.

In another series of experiments the influence of clonidine on the histamine-induced contraction was examined by comparing the histamine dose-response curves obtained before (control) and 10 min after addition of clonidine to the bath. The mechanisms involved in the potentiation observed were then investigated by means of different pharmacodynamic agents; a first histamine dose-response curve was established 15 min after addition of each individual agent and a second curve 15 and 10 min after addition of the agent and of clonidine, respectively.

Responses to clonidine and histamine were expressed as a percentage of the maximal response to histamine and −log EC50 values of histamine were defined as the negative log of the concentration that caused a 50% increase of maximal induced tension.

Drugs used were: clonidine HCl (Boehringer Ingelheim), prazosin HCl (Pfizer), propranolol HCl (ICI), histamine HCl (Prolabo), phentolamine methanesulphonate (Ciba-Geigy), reserpine (Ciba-Geigy), FPL 55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4*H*-chromene-2-carboxylate) (Fisons), indomethacin (MSD), nicotindole (Labaz), yohimbine HCl (Sigma), methysergide bimaleate (Sandoz), AR-C 239 HCl (2-(2-[4-(*O*-methoxyphenyl)piperazin-1-yl]-ethyl)-4,4-dimethyl-(2*H*,4*H*)-isoquinoline-1,3-dione HCl) (Karl Thomae), cocaine HCl (Prolabo).

All values quoted are means ± s.e.m. Statistical analysis of the results was performed using Student's *t*-test.

Results and discussion

Clonidine (10^{-6} to 3×10^{-4} M) produced concentration-dependent contractions in guinea-pig isolated tracheal spirals. This effect was strongly reduced by prazosin (3×10^{-7} M), but not significantly modified by yohimbine (3×10^{-7} M) (Fig. 1). Contrary to the results reported by Barnes et al (1983) with the dog isolated trachea

* Correspondence.

model, the maximal contracting effect of clonidine was modest when compared with the maximal contraction induced by histamine (Fig. 1).

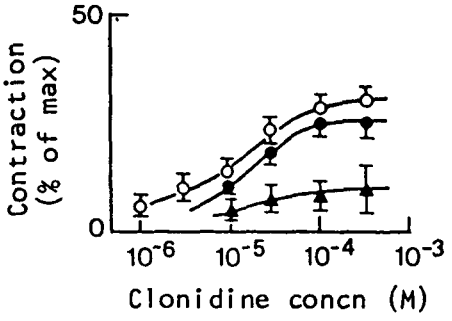

FIG. 1. Effect of clonidine on the guinea-pig isolated trachea under resting tone. Control (○). Pretreatment with prazosin 3×10^{-7} M (▲) or yohimbine 3×10^{-7} M (●).

We also found that clonidine (3×10^{-5} to 3×10^{-4} M) potentiated the effects of histamine ($2{\cdot}2 \times 10^{-7}$ to 2×10^{-5} M), as demonstrated by the displacement to the left of the histamine dose–response curves (Figs 2, 3). This potentiation was also reflected in the significant changes observed in histamine −log EC50 (0·47 ± 0·06 log unit) in the presence of clonidine in highest concentration (3×10^{-4} M) (Table 2).

Table 1. −log EC50 of histamine on the guinea-pig isolated trachea before and after pretreatment with clonidine. Experiments were performed on a group of 5 preparations.

	−log EC50 Histamine	Dose-responses curve shift to the left (log unit)
Control	5·28 ± 0·05	—
Clonidine 3×10^{-5} M	5·38 ± 0·11	0·10 ± 0·06
Clonidine 10^{-4} M	5·49 ± 0·13	0·21 ± 0·10
Clonidine 3×10^{-4} M	5·75 ± 0·06	0·47 ± 0·06*

* Significant shift to the left of the dose-responses curve to histamine ($P < 0{\cdot}01$).

Table 2. Effect of different pretreatments on the potentiation of histamine-induced contraction of the guinea-pig trachea by clonidine.

Pretreatment	n	−log EC50 of histamine: Before clonidine	−log EC50 of histamine: After clonidine	Dose-response curve shift to the left (log unit)
Control	5	5·28 ± 0·05	5·75 ± 0·06**	0·47 ± 0·06††
Indomethacin 10^{-6} M	4	5·21 ± 0·09	5·75 ± 0·08**	0·54 ± 0·04†
FPL 55712 10^{-6} M	4	5·32 ± 0·03	5·72 ± 0·09**	0·40 ± 0·10†
Nictindole 3×10^{-7} M	4	5·18 ± 0·12	5·75 ± 0·13*	0·57 ± 0·02†††
Yohimbine 3×10^{-7} M	4	5·34 ± 0·04	5·84 ± 0·08**	0·50 ± 0·07††
Nicardipine 10^{-7} M	4	5·25 ± 0·02	5·61 ± 0·08**	0·36 ± 0·06††
Prazosin 3×10^{-7} M	4	5·40 ± 0·02	5·47 ± 0·04	0·07 ± 0·03
AR-C 239 10^{-6} M	4	4·98 ± 0·11	5·17 ± 0·06	0·19 ± 0·06†

−log EC50 after clonidine signficantly different from −log EC50 before clonidine: *$P < 0{\cdot}05$; **$P < 0{\cdot}01$.

Statistical significance of the concentration-response curve shift to the left: †$P < 0{\cdot}05$; ††$P < 0{\cdot}01$; †††$P < 0{\cdot}001$.

Table 2 shows the influence on the histamine-clonidine interaction of pretreatment with seven pharmacodynamic agents. Since indomethacin, FPL 55712 or nictindole had no effect on the potentiation of the histamine-induced contraction by clonidine, a mechanism mediated by arachidonic acid derivatives could be ruled out. Similarly, the lack of effect of yohimbine or nicardipine showed that the potentiation was not due to stimulation of α_2-adrenoceptors or to the opening of calcium channels which is often associated with stimulation of these receptors in peripheral smooth muscles (Van Meel et al 1981; Godfraind et al 1982). On the other hand, the potentiating effect of clonidine was significantly reduced by AR-C 239 (10^{-6} M) and was suppressed by prazosin (3×10^{-7} M), which suggests that an α_1-adrenergic mechanism was involved. An α_1-adrenergic activity of clonidine in high doses has been reported with other organs (Schmitt et al 1968).

To conclude, clonidine in high concentration poten-

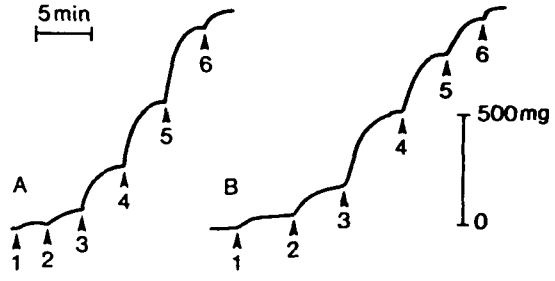

FIG. 2. Example of the action of clonidine (3×10^{-4} M) on the histamine-induced contraction of the guinea-pig isolated trachea (one preparation). A = control (histamine alone); B = histamine plus clonidine. Concentrations are: 1, $2{\cdot}2 \times 10^{-7}$ M; 2, $6{\cdot}6 \times 10^{-7}$ M; 3, $2{\cdot}2 \times 10^{-6}$ M; 4, $6{\cdot}6 \times 10^{-6}$ M; 5, $2{\cdot}2 \times 10^{-5}$ M; 6, $6{\cdot}6 \times 10^{-5}$ M.

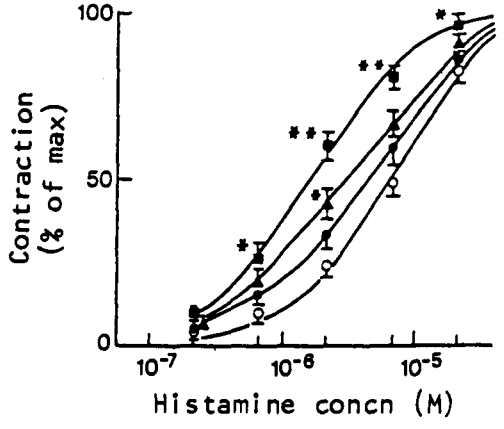

FIG. 3. Dose–response curves of histamine in the presence of clonidine in increasing concentrations. Control (○), clonidine 3×10^{-5} M (●), clonidine 10^{-4} M (▲), clonidine 3×10^{-4} M (■). Significant differences from control: *$P < 0{\cdot}05$; **$P < 0{\cdot}01$.

tiates the contracting effect of histamine on guinea-pig isolated trachea by stimulating α_1-adrenoceptors. The guinea-pig trachea therefore radically differs from the dog trachea which contracts when its α_2-adrenoceptors are stimulated by clonidine or noradrenaline (Barnes et al 1983).

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Prostaglandin D_2-induced potentiation of hexobarbitone hypnosis in rats: role of 5-hydroxytryptamine

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Prostaglandin D_2 (PGD_2) produced a dose-related increase in the duration and incidence of induction of sleep induced by hexobarbitone, in rats. Pretreatment with pharmacological agents known to reduce selectively brain 5-hydroxytryptaminergic activity, significantly inhibited PGD_2-induced potentiation of hexobarbitone, indicating that this potentiation is mediated by 5-hydroxytryptamine.

Considerable evidence now exists which makes it possible to assign a physiological role for prostaglandins (PGs) in the central nervous system (Wolfe & Coceani 1979). Wolfe (1976) suggested that PGs exert a modulatory influence on central neuronal activity. PG biosynthesis and release is known to be stimulated by catecholamines and 5-hydroxytryptamine (5-HT) (Wolfe 1976; Schaefer et al 1978). Likewise, PGs are reported to influence central catecholaminergic (Bergstrom et al 1973) and tryptaminergic (Debnath et al 1978; Bhattacharya 1982) activity. Until recently, only PGs of the E and F series were investigated in mammalian brain functions. However, it is now evident that there is considerable species variation in the distribution of central PGs and PGD_2 is by far the most dominant PG in rat and mouse brains, the levels of PGE_2 and $PGF_{2\alpha}$ being considerably lower (Abdel-Halim et al 1977). Recent studies indicate that, like PGE_1 (Bhattacharya et al 1976), PGD_2 has a sedative action in rodents and potentiates pentobarbitone sleeping time (Laychock et al 1980; Hollingsworth & Patrick 1984). PGE_1-induced potentiation of hexobarbitone hypnosis has been shown to be a 5-HT-mediated response (Bhattacharya et al 1976). The present study was designed to investigate the role of 5-HT in PGD_2-barbiturate interaction.

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Materials and methods

Male Wistar strain albino rats (120–180 g) were housed in colony cages at an ambient temperature of 25 ± 2 °C and fed on standard pellet chow with free access to water. Experiments were conducted at this ambient temperature between 0900 h and 1400 h. Food was withdrawn 18 h before and water just before the experiment.

Intracerebroventricular (i.c.v.) cannulation of the right lateral ventricle was performed in pentobarbitone sodium (40 mg kg^{-1} i.p.) anaesthetized rats (Feldberg & Lotti 1967). Experiments were conducted a week after the insertion of indwelling cannulae. All the drugs, except PGD_2 and hexobarbitone, were administered i.c.v. dissolved in 10 µl of artifical cerebrospinal fluid (csf). Control animals received an equivalent volume of artificial csf via the same route.

Two doses of hexobarbitone were used, one (100 mg kg^{-1} i.p.) which induced sleep in all rats, and the other (50 mg kg^{-1} i.p.) which had no detectable hypnotic effect. These two doses have been designated as the hypnotic and the sub-hypnotic dose of hexobarbitone, respectively. The sleeping time was measured as the interval between the loss and regaining of the righting reflex, with sufficient mobility to move beyond the border of a 12 in circle. In the groups in which the sub-hypnotic dose of hexobarbitone was used, percentage induction of sleep was the experimental criterion. No effort was made to assess the latency of the onset of hypnosis in either group. PGD_2, suspended in 1% ethanol was administered, after dilution with 0·9% NaCl (saline), in graded doses (0·2, 0·5 and 1 mg kg^{-1} i.p.) to groups of rats 15 min before hexobarbitone. Control animals received the equivalent volume of 1%