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Some actions of sodium nitroprusside and glyceryl trinitrate on guinea-pig isolated trachealis muscle

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The smooth muscle relaxant actions of sodium nitroprusside and glyceryl trinitrate have been compared to those of aminophylline and isoprenaline on isolated guinea pig trachealis muscle. Ethacrynic acid ($0{\cdot}25 \times 10^{-4}$ M), an alkylator of sulphydryl groups, interacted differently with the four agents. In the presence of ethacrynic acid the concentration response curve of the muscle preparation to sodium nitroprusside and glyceryl trinitrate was shifted to the higher concentration ranges and the maximum response was severely reduced. The concentration response curve for isoprenaline was shifted to the higher concentration ranges with no change in the maximum response and the response to aminophylline was unchanged. These results argue against common intermediate sites of action involving sulphydryl groups of the four agents in guinea-pig trachealis.

At the present time the clinical use of sodium nitroprusside and glyceryl trinitrate is related to their vasodilator properties. However, bronchodilator activity, at least in the case of sodium nitroprusside, has been investigated as another potential therapeutic use (Jamieson & Taylor 1979). In vascular smooth muscle, Needleman et al (1973) proposed that vasodilation occurred via an intermediate site which was common to isoprenaline, papaverine, theophylline, sodium nitroprusside, and glyceryl trinitrate. This hypothesis was based on the observation that the vascular relaxation produced by all these agents was prevented by ethacrynic acid, an alkylator of sulphydryl groups (Birkett 1973). The present study was designed to determine whether there was any evidence that a similar common pathway was involved in relaxation of trachealis muscle. Four smooth muscle relaxants were chosen which, according to Needleman's model, initiated relaxation by quite different mechanisms prior to the activation of the proposed common pathway. Isoprenaline, by activation of adenyl cyclase, and theophylline and papaverine, by inhibition of phosphodiesterase, increase cyclic 3,5-adenosine monophosphate concentrations in tissues. In contrast sodium nitroprusside has little affect on this cyclic nucleotide (Triner et al 1971). Both sodium nitroprusside and glyceryl trinitrate increase guanylate cyclase activity, but the relevance of this to smooth muscle relaxation is unknown (Murad et al 1979). There is evidence that sodium nitroprusside does not act through the 'nitrate receptor' postulated for glyceryl trinitrate and other vasodilator nitrates, as cross tachyphylaxis does not occur (Tinker & Michenfelder 1976).

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Methods

Female guinea-pigs, 350–450 g, were killed by stunning and bleeding. Tracheae were dissected out, cleaned and cut longitudinally through the cartilage ring opposite the trachealis. Small segments of trachea were mounted in a tissue bath containing Krebs-Henseleit solution at 37 °C and bubbled with 5% carbon dioxide in oxygen. The preparation was set up under a tension of 100 mg for isotonic recording using a Washington Type 2 transducer connected to a Rikadenki pen recorder (Jones 1978). Each tissue was exposed to 0·6 mM aminophylline for 10 min. Preliminary experiments had shown that this causes maximal relaxation of the preparation. After washing, the tissues regained spontaneous tone and a second maximal response to aminophylline was obtained. Subsequent relaxations of the preparation were expressed as a percentage of the second aminophylline maximum response (A_{max}). On separate preparations from the same trachea cumulative concentration response curves were obtained for either sodium nitroprusside, glyceryl trinitrate, isoprenaline or aminophylline. A 2·5 min contact time was allowed for each concentration of drug. This contact time was long enough for the relaxation after each addition of drug to begin to plateau but, in the case of isoprenaline and aminophylline, maximum relaxation was not achieved with every concentration. Despite this, the negative $\log_{10}$ EC50s for these two agents were similar to those obtained by others using a 15 min contact time (Foster 1966; Beckett & Foster 1972). After the tissue had regained its spontaneous tone with repeated washing the preparation was incubated with ethacrynic acid $0{\cdot}8 \times 10^{-4}$ to $6{\cdot}6 \times 10^{-4}$ M for 1 h. Control preparations were not exposed to ethacrynic acid. At the end of this period the tissue was washed six times over 1 h. Cumulative concentration response curves for the previously tested smooth muscle relaxant were obtained on the control and ethacrynic acid treated preparations. Thus the responses of the control preparations were time matched to the test preparation to account for any deterioration of responses due to time. Results are expressed as mean values ± s.e.m. of not less than six experiments.

Results

All four drugs caused a concentration-dependent relaxation of the trachealis muscle and were able to achieve the same maximal relaxation as seen with aminophylline (Fig. 1a–d).

Ethacrynic acid usually caused a small increase in tension of the tissue followed by relaxation, but the tension returned to pre-ethacrynic acid values after repeated washes. After incubation with ethacrynic acid ($0{\cdot}25 \times 10^{-4}$ M) responses to aminophylline were unchanged as were the responses to time matched control preparations (Fig. 1a). The log concentration response curve for isoprenaline was shifted to higher concentration ranges in a parallel fashion (Fig. 1b). The negative molar $\log_{10}$ EC50 for isoprenaline before ethacrynic acid was $7{\cdot}477 \pm 0{\cdot}142$ and after ethacrynic acid $6{\cdot}837 \pm 0{\cdot}113$ ($P < 0{\cdot}01$). The negative log EC50 for isoprenaline in time matched control preparations was $7{\cdot}315 \pm 0{\cdot}234$.

In contrast, the responses of the trachealis to sodium nitroprusside and glyceryl trinitrate were greatly reduced by ethacrynic acid incubation and the maximum relaxation was depressed (Figs 1c, d). Time matched control preparations showed no diminution in the maximum response to sodium nitroprusside and glyceryl trinitrate, indeed responses to glyceryl trinitrate were slightly enhanced. No diminution in the response to glyceryl trinitrate was seen even when the log concentration response curve was repeated four times. Smaller concentrations of ethacrynic acid ($0{\cdot}8 \times 10^{-4}$ to $1{\cdot}6 \times 10^{-4}$ M) had no significant effects on the responses to any of the bronchodilators and higher concentrations ($6{\cdot}6 \times 10^{-4}$ M) caused an irreversible loss of the spontaneous tone exhibited by the preparation.

Discussion

Using vascular smooth muscle, Needleman et al (1973) demonstrated that ethacrynic acid could inhibit the relaxant effects of all the vasodilators tested and concluded that relaxation of vascular muscle, however initiated, involved a common ethacrynic acid sensitive site. It is clear from the present study that relaxation of trachealis muscle can occur via a mechanism not involving an ethacrynic acid sensitive site as aminophylline induced relaxations were unaffected by ethacrynic acid treatment. Nevertheless ethacrynic acid did modify the responses of the trachealis muscle to isoprenaline, sodium nitroprusside and glyceryl trinitrate. The concentration response curve to isoprenaline was shifted to higher concentration ranges by $2{\cdot}5 \times 10^{-4}$ M ethacrynic acid and glyceryl trinitrate and sodium nitroprusside were unable to cause a maximal relaxation of the tissue.

As well as alkylating sulphydryl groups, ethacrynic acid has been shown to reduce adenosine triphosphate levels and ion flux due to general reduction in energy supply to the tissue (Daniel et al 1971). Concentrations of 10^{-3} M ethacrynic acid or more were required for this effect and thus this mechanism is unlikely to account for the interactions of ethacrynic acid with smooth muscle relaxants on the trachealis muscle. It may be relevant, however, to the irreversible loss of spontaneous tone of the trachealis muscle when exposed to high concentra-

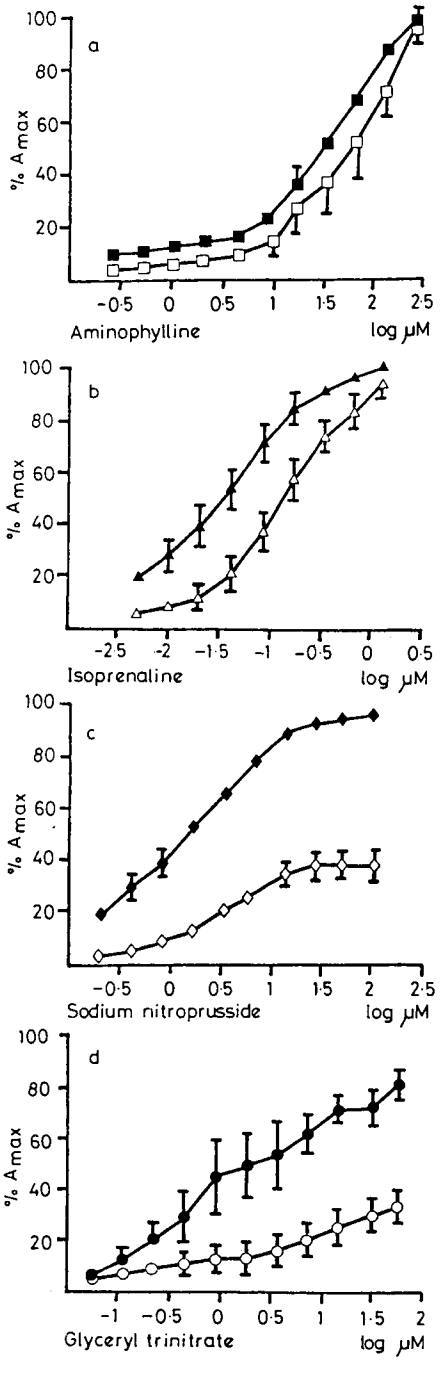

FIG. 1. Effects of incubation with $2{\cdot}5 \times 10^{-4}$ M ethacrynic acid (open symbols) on the responses of the trachealis muscle to: (a) aminophylline, (b) isoprenaline, (c) sodium nitroprusside, and (d) glyceryl trinitrate. Control responses are illustrated with closed symbols. Results are expressed as a % of the maximum response obtained to aminophylline (A_{max}) (means ± s.e.m., n = 6).

tions of ethacrynic acid. A significant decrease in titratable SH groups has been demonstrated with 1×10^{-4} M ethacrynic acid in the rabbit aorta (Needleman et al 1973) and thus it is likely that 2.5×10^{-4} M ethacrynic acid will have similar effects on trachealis muscle. In view of this it is probable that alkylation of sulphydryl groups is responsible for the interactions reported in this study. Needleman et al (1973) suggested that sodium nitroprusside and glyceryl trinitrate acted on primary receptors involving SH groups as well as the 'common intermediate site' also affected by isoprenaline. This may explain the differences in the effects of ethacrynic acid on the responses of the trachealis to isoprenaline on the one hand and glyceryl trinitrate and sodium nitroprusside on the other.

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Prolongation of thiopentone-induced sleep by trazodone and its metabolite, *m*-chlorophenylpiperazine

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Trazodone and its metabolite, *m*-chlorophenylpiperazine (CPP) prolonged significantly thiopentone-induced sleep in mice. Neither trazodone, nor CPP changed the cerebral concentrations of thiopentone. As cyproheptadine by itself did not affect thiopentone sleep and did not antagonize the effect of CPP, the effect of trazodone and CPP seems to be independent of their respective 5-HT-antagonistic and 5-HT-agonistic properties.

Interaction of drugs with barbiturates, measured as their influence on barbiturate sleeping time, is an old and commonly used test which is supposed to detect a general depressant action of a drug. However, the precise nature of prolongation of barbiturate sleeping time may differ from one drug to another, and may often be unknown. It may depend both on the kind of barbiturate and the mechanism of action of the tested drug. Thus, tricyclic antidepressants prolong the barbiturate sleeping time (see Gyermek 1966), but the interaction of these drugs with barbitone was ascribed to an increased CNS sensitivity to barbiturates, while the prolongation of pentobarbitone sleep was considered to result from inhibition of the hepatic metabolism of the barbiturate (Liu et al 1975). More recently, prolongation of thiopentone sleeping time by desipramine was ascribed to an interaction of desipramine with the noradrenergic system (Mason & Angel 1983).

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Trazodone, an atypical antidepressant drug (Silvestrini 1982), also prolongs barbiturate sleep (Silvestrini et al 1968), but the mechanism of this action remains unknown. The pharmacological profile of trazodone is dissimilar from that of other antidepressants (Maj 1978; Silvestrini 1982) and the drug seems to be devoid of a direct influence on the noradrenergic system but to interact in a complex manner with the brain 5-hydroxytryptamine (5-HT) system (Maj et al 1979). The complexity is caused by the fact that trazodone, which has central and peripheral anti-5-HT properties (Baran et al 1979; Maj et al 1979; Hingten et al 1984), is biotransformed with the formation of *m*-chlorophenylpiperazine (CPP) (Melzacka et al 1979; Caccia et al 1981) which has 5-HT-mimetic properties (Samanin et al 1979; Rokosz-Pelc et al 1980; Fuller et al 1981). We aimed to assess if trazodone and CPP have a similar effect on thiopentone sleeping time, if this effect is related to their 5-HT-antagonistic or 5-HT-mimetic properties, and if the two drugs change the cerebral levels of the barbiturate.

MATERIALS AND METHODS

Male mice of the CD-1, Albino-Swiss or C57BL/6 strain were kept under standard animal room conditions, with free access to food and water, for at least one week before the experiment.