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EFFECTS OF GLYCERYL TRINITRATE AND SIN-1A ON MUSCULATURE AND VASCULATURE OF THE DOG TRACHEA *in situ*

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In dog trachea *in situ*, perfused arterially with blood, isoprenaline, glyceryl trinitrate and N-nitroso-*N*-morpholinoamino-acetonitrile (SIN-1A) given intra-arterially, decreased tracheal tone which had been elevated by neostigmine and increased tracheal blood flow. Glyceryl trinitrate and SIN-1A were more effective on the tracheal musculature than on the tracheal vasculature whereas isoprenaline was equally effective on both.

Introduction Organic nitrates used as antianginal drugs because of their ability to relax vascular smooth muscle are also known to relax bronchial smooth muscle (Needleman & Johnson, 1980). Glyceryl trinitrate and erythrityl tetranitrate have been reported to be effective, like isoprenaline, in relieving bronchial asthma (Hirshleifer & Arora, 1961). Pentaerythrityl tetranitrate when inhaled by anaesthetized dogs reduces pulmonary resistance, and in this respect is one fifth to one third as potent as isoprenaline (Aviado, Kishimoto & Kneidinger, 1969). The potencies of glyceryl trinitrate and isoprenaline in relaxing air-way smooth muscle or the relative effectiveness of glyceryl trinitrate on airway and on blood vessels supplying the airways have not been investigated. We designed the present experiments to obtain such information. For this purpose we used the blood-perfused dog trachea *in situ* (Himori & Taira, 1976). In the present experiments we also investigated the effect of N-nitroso-*N*-morpholinoamino-acetonitrile (SIN-1A), an active metabolite thought to mediate the action of the syndonimine vasodilator, molsidomin (Kikuchi, Hirata, Nagaoka & Aramaki, 1970), since SIN-1A has been shown to resemble glyceryl trinitrate (Hashimoto, Taira, Hirata & Kokubun, 1970).

Methods Twelve mongrel dogs of either sex (10 to 15 kg), anaesthetized with pentobarbitone sodium (30 mg/kg, i.v.) were used. The tracheal vascular bed *in situ* was perfused through both cranial thyroid arteries with blood from the femoral artery. Perfusion pressure was kept constant at a value slightly higher than the mean systemic arterial pressure by the use of a Starling pneumatic resistor. Blood flow through the cranial thyroid arteries (tracheal blood flow) was measured with an electromagnetic flowmeter. A tracheal tube with a water-filled cuff attached was introduced into the trachea. Hydraulic pressure in the cuff was measured with a pressure transducer as intraluminal pressure of the trachea. Details of the preparation have been given previously (Himori & Taira, 1976). The cuff was filled initially with water to give a resting intraluminal pressure of about 40 cmH_2O, and during the experiment, to obtain increased tracheal tone, 3 μg of neostigmine methyl sulphate (Shionogi) was injected intra-arterially at about 30 min intervals. (−)-Isoprenaline hydrochloride (Sigma), glyceryl trinitrate (Nihon Kayaku) and N-nitroso-*N*-morpholinoamino-acetonitrile (SIN-1A, Takeda) were used. Isoprenaline and SIN-1A were dissolved in 0.9% w/v NaCl solution (saline) at a concentration of 1 mg/ml. Glyceryl trinitrate was dissolved in distilled water at a concentration of 617 μg/ml. All drugs solutions were diluted with saline and injected intra-arterially in a volume of 30 μl in 4 s. Doses of isoprenaline refer to the base. Values are given as mean ± s.e. mean, except where otherwise stated. Dose-response curves were treated as linear regressions and parallelism of the curves was analysed.

Results The mean tracheal blood flow was 5.6 ± 1.1 ml/min at the mean perfusion pressure of 137 ± 19 (s.d.) mmHg (n = 12). The mean systemic arterial pressure was 119 ± 6 mmHg. The mean basal tracheal tone raised by repeated injections of 3 μg of neostigmine was 60 ± 4 cmH_2O (n = 12).

Isoprenaline (0.03 to 0.3 μg), glyceryl trinitrate (0.1 to 3 μg) and SIN-1A (0.3 to 10 μg) all increased blood flow (vasodilatation) and decreased tone (dilatation) in the trachea in a dose-dependent manner (Figure 1). In high doses all three drugs abolished tone in the trachea. The dose-response curves for the three drugs for tracheal vasodilatation were parallel ($P < 0.05$). Glyceryl trinitrate and SIN-1A were about 32 and 220 times respectively less potent than isoprenaline on a weight for weight basis. The dose-response curves to the three drugs for tracheal dilatation were also all parallel ($P < 0.05$), and glyceryl trinitrate and SIN-1A were about 9 and 34 times respectively less potent than isoprenaline on a weight for weight basis. The selectivity for the tracheal musculature over the tracheal vasculature, determined from the ratio of the dose required to

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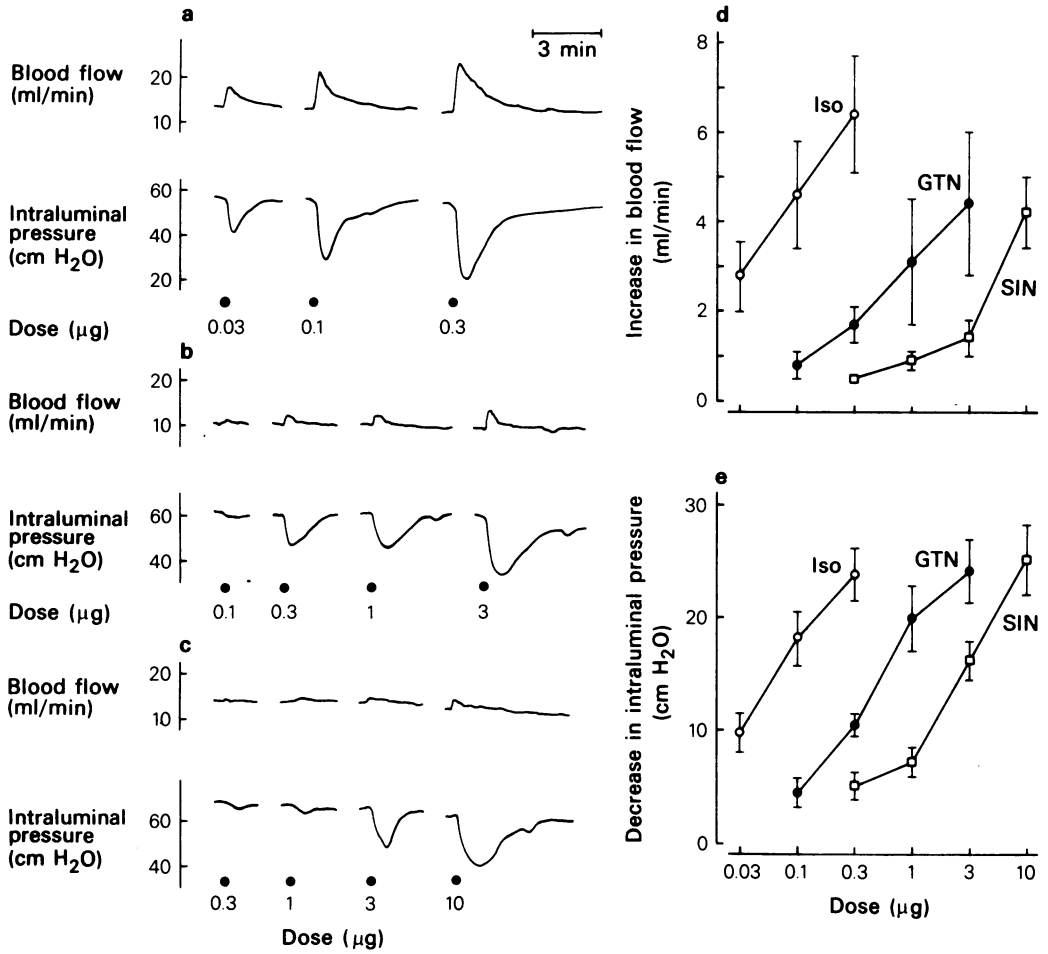

Figure 1 Responses of the tracheal vascular bed (blood flow rate) and musculature (intraluminal pressure) to (a) isoprenaline, (b) glyceryl trinitrate and (c) SIN-1A. (d) Dose-response curves for the increase in tracheal blood flow produced by isoprenaline (Iso), glyceryl trinitrate (GTN) and SIN-1A (SIN). (e) Dose-response curves for the decrease in tracheal intraluminal pressure. Each point represents the mean value and vertical bars show s.e. mean. The number of dogs is 6 for isoprenaline and SIN-1A; 5 for glyceryl trinitrate.

increase tracheal blood flow by 4 ml/min to that to decrease tracheal tone by 15 cmH_2O, was 1 for isoprenaline, 4 for glyceryl trinitrate and 7 for SIN-1A. The latter two drugs were more selective for tracheal musculature than for tracheal vasculature as compared to isoprenaline.

Discussion In the dog trachea *in situ*, glyceryl trinitrate and SIN-1A, like isoprenaline, relaxed tracheal musculature and abolished tracheal tone in high doses. Unexpectedly, glyceryl trinitrate and SIN-1A were more selective for the tracheal musculature than for vasculature than was isoprenaline. This contrasts with the tracheal dilator effect of calcium-antagonistic drugs e.g. nifedipine and verapamil which was much weaker than their tracheal vasodilator action (Himori & Taira, 1980). Thus, the mechanism of action of nitrates and nitroso compounds in relaxing tracheal smooth muscle, in which tone was increased by neostigmine, may be an inhibition of the release of calcium from intracellular storage sites, facilitation of the uptake of calcium by these stores, or the extrusion of calcium to extracellular space, rather than the inhibition of transmembrane influx of calcium. Indeed calcium influx may not play a major role in maintaining spontaneous tone (Himori & Taira, 1980) or in producing the contraction obtained in response to high concentrations of acetylcholine (Coburn, 1977; Farley & Miles, 1978) in tracheal smooth muscle. In the present experiments the increase in tracheal tone produced by 3 μg of neo-stigmine was

greater than that produced by intra-arterial infusion of acetylcholine at a rate of 100 μg/min (unpublished observations). Therefore, in the present experiments the concentration of endogeneous acetylcholine may have been high. The present results suggest that organic nitrates and nitroso compounds would be effective in the treatment of bronchial asthma.

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