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Effect of frusemide on the reactivity of rat portal vein

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Short lengths of rat portal vein were suspended in Krebs bicarbonate solution. Contractile responses to noradrenaline and angiotensin II and the parameters of spontaneous contractions were determined before and after addition of frusemide at 10^{-6} and 10^{-4} g/ml. Frusemide at 10^{-6} g/ml slightly suppressed the amplitude of spontaneous contractions and slightly decreased the responses to noradrenaline and angiotensin II. At 10^{-4} g/ml the drug had a much greater inhibitory effect on endogenous and exogenous stimulation. The concentrations used appear to be similar to those achieved during effective diuretic and hypotensive treatment. It is postulated that frusemide may have a direct inhibitory action on the reactivity of vascular smooth muscle particularly in the capacitance vessels.

The persistent hypotensive effect of diuretic agents despite restoration of plasma volume and cardiac output seems to be due, at least in part, to a direct effect on vascular resistance (Conway & Palmero, 1963), and there are many reports that the thiazide diuretics interfere with the pressor activity of noradrenaline *in vivo* (Freis, Wanko & others, 1960; Mendlowitz, Naftchi & others, 1960; Feisal, Eckstein & others, 1961; Jackson & Duff, 1963) and perhaps *in vitro* (Rubin, Beauregard & Freeman, 1960). Conway & Palmero (1963) concluded that chlorothiazide exerts its effect by changes more complex than mere loss of body water, probably by changes in distribution of water or electrolytes affecting vascular smooth muscle.

We have examined the effect of frusemide, a diuretic with hypotensive action, on the contractile activity of rat isolated portal vein responding to endogenous or exogenous stimulation.

METHODS

Male Sprague-Dawley rats (150–300 g) were killed and the portal vein was removed and suspended vertically in a 10 ml organ bath containing Krebs - bicarbonate solution at 37° bubbled with a gas mixture of 5% carbon dioxide in oxygen (Umbreit, Burris & Stauffer, 1964). A passive tension of 400–600 mg was applied, and contractions were measured with a Grass force-displacement transducer coupled to an Offner dynograph. Agonist drugs were injected in 0·1 ml volumes of Krebs directly into the organ bath and were washed out immediately after the response was maximal. Additions of angiotensin II (val - 5 - angiotensin II amide, Hypertensin Ciba) were made at intervals of 10 min to avoid tachyphylaxis. Noradrenaline (Noradrenaline bitartrate, Levophed, Winthrop) doses were also spaced 10 min apart. The spontaneous contractions, or the responses to noradrenaline or angiotensin II, were measured during at least 1 h in normal medium and then in medium containing frusemide (Lasix, Hoechst) at 10^{-6} or 10^{-4} g/ml for periods of 15–150

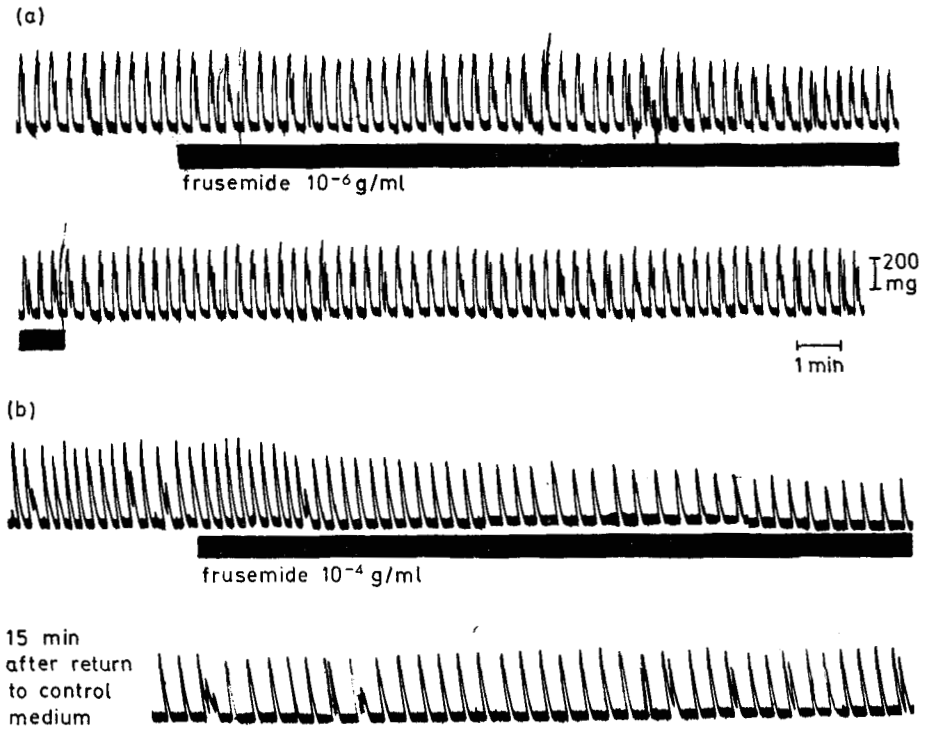

FIG. 1. The effect of frusemide on spontaneous contractions of rat portal vein. Frusemide was included in the Krebs - bicarbonate solution during the periods indicated by the bar. (a) top panel, 10^{-6} g/ml, (b) bottom panel, 10^{-4} g/ml.

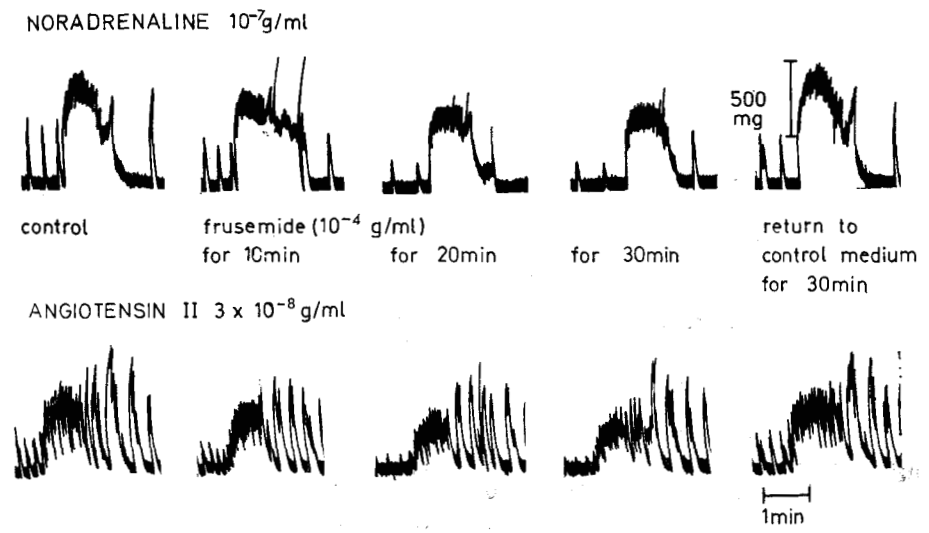

FIG. 2. The time-course of effect of frusemide (10^{-4} g/ml) on responses to medium-effective concentrations of noradrenaline and angiotensin II.

min. After this time, the medium was changed back to normal Krebs solution for observations during a recovery period.

RESULTS

Exposure of the rat portal vein to 10^{-6} g/ml frusemide (5 experiments) caused a gradual decrease in the amplitude of spontaneous contraction (Fig. 1a). During exposure to 10^{-4} g/ml of drug (18 experiments), the amplitude of contraction fell progressively, so that after 10 min it was reduced to approximately 60% of the control response (Fig. 1b). The amplitude gradually returned to the control value after the drug had been washed out. Frusemide did not consistently affect the frequency of spontaneous contractions.

Frusemide at 10^{-6} g/ml caused a slight decrease in contractile responses to medium-effective concentrations of noradrenaline and angiotensin II (4 experiments); 10^{-4} g/ml frusemide substantially reduced responses to both noradrenaline (4 experiments) and angiotensin II (4 experiments). Typical examples are shown in Fig. 2.

Table 1 shows dose-response relations for noradrenaline (4 experiments) and angiotensin II (2 experiments) in normal Krebs solution and in Krebs solution containing 10^{-4} g/ml frusemide. Each response value is the mean of three estimates. Responses to each agent were reduced at all effective doses. Maximum responses to noradrenaline were suppressed on the average to 75% of control and maximum responses to angiotensin II in the presence of frusemide averaged 48% of control.

Table 1. *Effect of frusemide* (10^{-4} *g/ml*) *on responses to noradrenaline and angiotensin II.* (Results expressed as percentage of maximum response)

Agent	Medium	10^{-9}	10^{-8}	10^{-7}	10^{-6}	3×10^{-6}	10^{-5}
Noradrenaline	Krebs	0	13	57	82	100	99
	Krebs + frusemide	0	3	26	51	73	77
	Krebs	0	16	43	80	97	100
	Krebs + frusemide	0	5	27	66	76	74
	Krebs	0	23	62	94	100	100
	Krebs + frusemide	0	5	26	68	87	—
	Krebs	0	29	64	88	—	100
	Krebs + frusemide	0	19	40	69	—	73
	$\bar{X}$ Krebs	0	20·3	56·5	86·0	99·0	99·8
	$\bar{X}$ Krebs + frusemide	0	8·0	29·8	63·5	78·7	74·7
	s.e. Krebs	0	3·59	4·73	3·16	1·00	0·25
	s.e. Krebs + frusemide	0	3·69	3·42	4·21	4·26	1·20
	Significance of Difference (paired *t*-test)	—	0·05	0·05	0·05	N.S.	0·05
Angiotensin II	Krebs	0	43	82	100	100	
	Krebs + frusemide	0	4	35	38	33	
	Krebs	2	21	74	100	100	
	Krebs + frusemide	0	5	54	67	63	

DISCUSSION

Frusemide partially inhibited contractile responses of the rat portal vein to endogenous and to exogenous stimuli. Angiotensin II and noradrenaline stimulate via separate receptor sites in this preparation (Blair-West, McKenzie & McKinley, 1971) and these excitatory mechanisms are almost certainly independent of spontaneous myogenic excitation. Therefore, antagonism by frusemide is not due to chemical interaction with endogenous mediators or their pharmacological receptors.

Daniel & Nash (1965) reported that benzothiadiazines, diazoxide, and related diuretics did not affect sodium and potassium transport in isolated smooth muscle preparations, but more recently Daniel (1967) has shown that frusemide inhibits sodium extrusion in sodium-rich rabbit uterus and aorta. Frusemide inhibits renal reabsorption of sodium, mainly in the ascending limb of the loop of Henle (Seldin, Eknoyan & others, 1966; Bennett, Clapp & Berliner, 1967; Clapp & Robinson, 1968) and reduces entry of sodium into the cells of frog-skin (Nagel & Karger, 1964). This evidence suggests that frusemide may act on vascular smooth muscle by changing intracellular ionic concentrations, to modify excitation - contraction coupling or the contractile mechanism.

Daniel (1967) found that a frusemide concentration of 1·2–12 mg/ml was required to affect ionic transport in segments of rabbit aorta and uterus. These doses appear to be very high relative to effective intravenous diuretic doses of 0·5–10 mg/kg in man and experimental animals (Davidov, Kakaviatos & Finnerty, 1967; Muth, 1968; Daniel, 1967; Vander & Carlson, 1969). Assuming the drug is dispersed in extracellular fluid and slowly metabolized, concentrations of 2·5–50 mg/litre might be expected during diuretic therapy. Daniel concluded that the *in vitro* concentrations which affected ionic transport appeared to be too high to account for the hypotensive effect of frusemide. The concentrations shown to be effective in reducing contractility of rat portal vein, in the present study, are much lower than those effective on ionic transport in Daniel's study. A measurable reduction of amplitude of spontaneous contraction was caused by 1 mg/litre and 100 mg/litre caused substantial suppression of exogenous and endogenous stimulation. These values are near to those expected with effective diuretic doses and this tends to sustain the argument that depression of contractility of vascular smooth muscle cells may contribute to the hypotensive action of frusemide.

Although Daniel & Nash (1965) were unable to correlate hypotensive actions of diuretics with direct inhibition of *arterial* muscle contractility, it is possible that an effect is exerted, particularly on the *capacitance elements* of the circulation. The present data demonstrating depression of isolated portal venous smooth muscle by frusemide offer support for this possibility, whether or not it is mediated by effects on membrane ionic transport.

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