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Effects of Quinidine on Venous Responses to Adrenergic and Nonadrenergic Constrictor Stimuli: Indirect Evidence of Two Sites of Action in Vascular Smooth Muscle[1] (38116)

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Quinidine has a potent hypotensive action which is mediated in part by direct vasodilatation and inhibition of adrenergic constrictor tone in resistance vessels (1). The present experiments were done to characterize effects of quinidine on a venous bed and to investigate further the specificity of the inhibitory effects of quinidine on responses to venoconstrictor stimuli.

Methods. Twelve male mongrel dogs weighing 13–27 kg were anesthetized with chloralose (60 mg/kg iv) and urethane (600 mg/kg iv), ventilated artificially with a respirator, and treated with decamethonium bromide (0.3 mg/kg iv) and heparin (500 USP units/kg iv). The dorsal branch of the left lateral saphenous vein was cannulated at the ankle and perfused at constant flow (3 ml/kg/min) with blood from a femoral artery using a peristalic pump. Systemic arterial and venous perfusion pressures were measured using Statham P23AA tranducers and were recorded continuously on a Beckman RM Dynograph. At constant flow, increases in perfusion pressure indicated constriction in the isolated perfused segment of vein (2).

In six dogs, constrictor interventions were: sciatic nerve stimulation (supramaximal voltage, 10 msec square wave impulses) 1.5, 3, and 6 Hz; phenylephrine hydrochloride (Winthrop Laboratories, New York) 12, 24, 48 μg; 5-hydroxytryptamine (General Biochemicals, Chagrin Falls, Ohio) 1, 2, and 4 μg. In six different dogs, constrictor interventions were: l-norepinephrine (Levophed, Winthrop, N.Y.) 0.25, 0.5 and 1 μg base; and KCl, 0.75, 1.5 and 3 mEq. In 2 other dogs, pretreated with phenoxybenzamine (Dibenzyline, Smith, Kline and French, Philadelphia), 5 mg/kg iv, which inhibited totally responses to norepinephrine, venoconstrictor responses to KCl, 1.5 and 3 mEq were observed. This was done to eliminate the possibility that responses to KCl resulted from release of norepinephrine from sympathetic nerves. Drugs were diluted in 5% dextrose and water and injected in volumes of 0.1–0.2 ml into perfusion tubing upstream to the pump. These volumes of 5% dextrose in water alone did not produce changes in perfusion pressures. Potassium chloride was injected in volumes of 0.25–1 ml. Comparable injections of sodium chloride, 0.75–3 mEq (0.25–1 ml) had negligible effects on perfusion pressures.

Responses to constrictor interventions were observed in: (1) a control period after intravenous administration of the vehicle alone; (2) after intravenous administration of quinidine, 2.5 or 5 mg base/kg; and (3) again in a recovery period after waiting 30 min. The dose of quinidine (Quinidine Gluconate, Lilly, Indianapolis, Indiana) was diluted in 10 ml of 5% dextrose and water. Comparable

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amounts of 0.25% phenol were diluted in the same volumes to obtain the vehicle which represents the diluent for commercial Quinidine Gluconate.

Comparisons were made using a parallel line bioassay as a statistical test (3).

Results. In one group of 6 dogs, quinidine (2.5 mg base/kg) lowered mean arterial blood pressure from 116 ± 7.6 (±SE) mm Hg to 97 ± 10 mm Hg. In another group of 6 dogs, quinidine (5 mg base/kg) lowered the mean arterial pressure from 139 ± 5.4 mm Hg to 104 ± 4.5 mm Hg. Baseline venous perfusion pressures for each group were unchanged after quinidine (55 ± 10 mm Hg vs 51 ± 14 mm Hg before and after quinidine respectively for the first group of dogs; 43 ± 2.4 vs 42 ± 2.4 mm Hg in the second group).

Quinidine (5 mg base/kg) reduced significantly ($P < 0.05$) constrictor responses to postganglionic sympathetic nerve stimulation and phenylephrine in the saphenous vein, but responses to the non-adrenergic agonist, 5-hydroxytryptamine, were not altered (Fig. 1A and Table I).

In a separate group of 6 dogs, quinidine (2.5 mg base/kg) reduced constrictor responses to norepinephrine and KCl, but responses to norepinephrine were inhibited significantly more ($P < 0.05$). Relative potency calculations indicated that norepinephrine was inhibited by a factor of 5 (relative potency, 0.19, upper and lower 95% confidence limits, 0.30 and 0.08 respectively) whereas KCl was inhibited by a factor of 2 (relative potency, 0.48, upper and lower 95% confidence limits, 0.59 and 0.38, respectively). The fact that 95% confidence limits for norepinephrine and

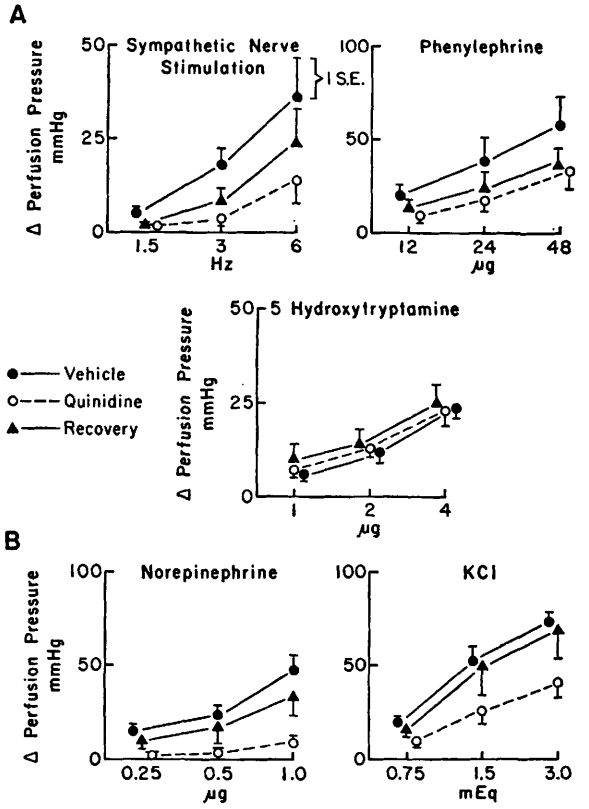

FIG. 1. Responses in perfused saphenous vein to constrictor stimuli after intravenous administration of vehicle (o—o), quinidine, (0—0), and a 30 min recovery period (△—△). Each point is the average of observations on 6 animals ± 1SE. A and B represent different groups of animals. In animals of group A, the dose of quinidine was 5 mg base/kg; in group B, the dose was 2.5 mg base/kg.

TABLE I. Relative Potency of Quinidine's Inhibitory Effects.[a]

	RP	95% Confidence Limits	
		Lower	Upper
SNS	0.28	0.06	0.60[b]
PE	0.30	0.07	0.62[b]
5-HT	1.04	0.71	1.55

[a] Responses after quinidine were compared to responses after vehicle using analysis of variance and a parallel line bioassay as a statistical test (3). Relative Potency (RP) and 95% confidence limits indicate the ratio (and limits for the ratio) of a stimulus which given a certain response after vehicle to the stimulus which gives the same response after quinidine.

[b] Responses to sympathetic nerve stimulation (SNS) and phenylephrine (PE) were considered to be inhibited significantly ($P < 0.05$) after quinidine if a potency ratio of 1.0 was not included within the confidence limits. Values for RP indicate that responses to adrenergic stimuli were inhibited by a factor of three after quinidine. In contrast, responses to 5-hydroxytryptamine (5-HT) were not inhibited. The difference between adrenergic stimuli (SNS and PE) and 5-HT is emphasized by the fact that 95% confidence limits do not overlap.

KCl do not overlap indicates that inhibition of responses to norepinephrine was significantly greater than inhibition of responses to KCl (3).

In two dogs pretreated with phenoxybenzamine (5 mg/kg iv) which blocked responses to norepinephrine completely, quinidine still produced almost a two fold inhibition of responses to KCl (Fig. 2). Before phenoxybenzamine, increases in venous perfusion pressure in response to KCl, 1.5 and 3 mEq, were 24 and 58 mm Hg respectively; corresponding increases after phenoxybenzamine and before quinidine were 14 and 48 mm Hg. After quinidine (5 mg/kg) corresponding increases were only 8 and 26 mm Hg.

After a 30 min recovery period the responses to all constrictor agonists tended to return to the control values.

Discussion. These results demonstrate that quinidine inhibits constrictor responses to adrenergic stimuli in a venous bed in the canine hindlimb. Inhibition occurred after doses of 2.5 and 5 mg quinidine base/kg, doses which in a previous study produced serum quinidine levels of about 2–5 mg/liter (1). These quinidine levels are within the usual therapeutic range.

Inhibition of adrenergic vasoconstrictor tone in resistance vessels contributes to hypotension after quinidine (1). The present results suggest that blockade of adrenergic vasoconstriction occurs also in capacitance vessels and raises the possibility that this action in addition to the action on resistance vessels might contribute to hypotension after administration of quinidine.

Inhibition of venoconstrictor responses to postganglionic sympathetic nerve stimulation,

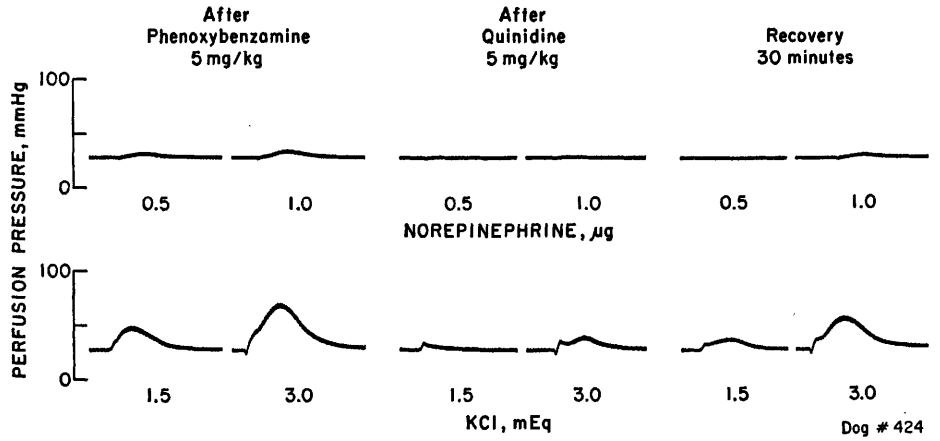

FIG. 2. Quinidine inhibited the venoconstrictor response to KCl in saphenous vein after pretreatment with phenoxybenzamine indicating that blockade of KCl did involve α adrenergic mechanisms.

phenylephrine and norepinephrine cannot be explained by an effect of quinidine on baseline venous resistance since baseline perfusion pressures and therefore baseline resistances were not altered by administration of the drug.

A nonspecific depression of constrictor responsiveness with time is excluded by observations that responses to all agonists tended to return toward control values during the recovery period. Furthermore, venoconstrictor responses to 5-hydroxytryptamine were not inhibited indicating that there was some specificity to blockade of adrenergic stimuli. Inhibition also cannot be attributed to the diluent for commercial quinidine which is 0.25% phenol since this was present in the vehicle as well as quinidine.

Phenylephrine is inactivated minimally and very slowly by uptake into sympathetic nerve terminals (4) so that the responses to this drug reflect primarily accessibility of alpha adrenergic receptors and vascular smooth muscle reactivity. Comparable inhibition of nerve stimulation and phenylephrine in the same dogs suggests that decreased availability of adrenergic receptors accounted completely for the observed reduction in response to nerve stimulation. There is no need to postulate changes in the function of sympathetic nerve terminals to explain the present results, although, of course, such changes cannot be excluded totally.

In the present experiments, constrictor responses to KCl as well as norepinephrine were reduced significantly by quinidine. However, responses to norepinephrine were reduced significantly more. These two interventions were given to the same dogs. Since the order of administering interventions was varied, a lesser reduction in the response to KCl cannot be explained by waning quinidine effect. Also considered was the possibility that KCl produced venoconstriction by triggering release of norepinephrine from sympathetic nerves so that quinidine's effect on KCl might be mediated by adrenergic mechanisms. This possibility was investigated in 2 dogs pretreated with phenoxybenzamine. Despite complete inhibition of responses to norepinephrine and some reduction in responses to KCl, KCl still produced substantial venoconstriction. This is consistent with previous reports that phenoxybenzamine inhibits slightly constrictor effects of KCl on isolated preparations of aorta (5, 6). With adrenergic contributions to constrictor effects of KCl eliminated by phenoxybenzamine, subsequent administration of quinidine still produced approximately a two fold reduction in responses to KCl. These observations in conjunction with more pronounced inhibition of norepinephrine than KCl suggests that quinidine might possibly act at 2 sites in vascular smooth muscle. One site would appear to involve adrenergic receptors; the other site would appear to be activated by potassium. This speculation is supported by previous work (7–10) which indicates that KCl and norepinephrine produce vasoconstriction by different mechanisms. Potassium appears to influence a more labile, accessible pool of calcium which may be extracellular and/or loosely and superficially bound. Norepinephrine appears to influence a more tightly bound fraction of the calcium pool which may be located intracellularly. There is precedent for speculating that intracellular calcium might be involved. Quinine which is the optical isomer of quinidine inhibits binding and uptake of calcium by isolated vesicles of sarcoplasmic reticulum (11). However, the present results do not directly confirm that quinidine inhibits a constrictor stimulus by influencing intracellular calcium movement. Inhibition of norepinephrine could be explained as well by steric hindrance of norepinephrine binding with alpha adrenergic receptors on smooth muscle cells. The exact mechanisms of the inhibitory actions of quinidine require further investigation, but at least 2 sites of action are suggested by these observations.

Summary. The effect of quinidine on adrenergic and nonadrenergic constrictor stimuli was studied in the perfused canine saphenous vein. Quinidine significantly reduced constrictor responses to sympathetic nerve stimulation, phenylephrine and norepinephrine while responses to a nonadrenergic constrictor drug, 5-hydroxytryptamine, were not reduced, indicating that quinidine interferes with stimuli that activate alpha adrenergic receptors. Quinidine also had a smaller yet significant effect on constrictor response to KCl suggesting a possible second site of action.

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