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Effect of Quinidine upon the Vascular Response to Norepinephrine in the Dog Forelimb (40772)

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Quinidine is a commonly used antiarrhythmic agent which can produce cardiodepression and vasodilation in large doses or in a hypersensitive subject (1). It is reported to inhibit Mg^{2+}-ATPase (2), Na^+,K^+-ATPase (2–5) and Ca^+-ATPase (2, 6–8) activities in various tissues and concentrations. We previously showed that ouabain, a potent Na^+,K^+-ATPase inhibitor, blocks potassium vasodilation in several vascular beds (9, 10). Since quinidine is also a Na^+,K^+-ATPase inhibitor we wondered whether it would also block K^+ vasodilation. Since the other two enzymes are Mg^{2+} and Ca^{2+} sensitive the effect of quinidine on magnesium vasodilation and calcium vasoconstriction was also examined. Norepinephrine and acetylcholine were administered as control substances. We found that quinidine in therapeutic concentrations had little effect on the responses to the cations and acetylcholine but produced rather dramatic changes on the slow phase response of norepinephrine. This paper presents these findings.

Methods. The preparation was the innervated dog forelimb perfused at constant flow with arterial blood. Adult mongrel dogs of either sex were anesthetized with sodium pentobarbital and ventilated artificially. The right brachial artery, brachial vein, cephalic vein, and nerves were dissected free of surrounding tissue above the elbow and tourniquets placed around the rest of the soft tissues to minimize collateral flow. The animals were heparinized and the proximal femoral and distal brachial arteries connected with a length of polyethylethylene tubing. A pulsatile, pressure-independent blood pump was interposed along this tubing so that the brachial artery and forelimb could be perfused at constant flow. The forelimb perfusion pressure was recorded from the brachial cannula just upstream to its entry into the brachial artery. Systemic arterial blood pressure was recorded from the brachial artery central to the entry of the perfusion cannula. Blood flow to the forelimb was set at a constant 100 ml/minute which produced a perfusion pressure slightly less than systemic arterial blood pressure. All agents were infused or injected in a bolus into the forelimb by way of the femoral-brachial tubing, upstream to the pump to assure adequate mixing.

Two groups were studied. One group of 10 animals received an infusion of quinidine solution at rates of 0.494, 1.23 and 2.47 ml/minute. The solution contained 500 μg/milliliter quinidine hydrochloride; the osmolality was brought to 300 mOsm/kg with sodium chloride. The three infusion rates produced calculated plasma concentrations for quinidine base of 6, 15, and 31 μM. A second group of five animals received saline at the same volume rates. Prior to the infusion of either quinidine or saline, KCl, $CaCl_2$, $MgCl_2$, acetylcholine, and norepinephrine were administered in that order. The KCl, $CaCl_2$, and $MgCl_2$ were made up as isosmotic solutions (150, 200, and 200 meq/liter, respectively) and injected in a 1-ml bolus. Acetylcholine chloride and norepinephrine (levarterenol bitartrate) were placed in saline and given in 0.1-ml boluses containing 1.0 and 0.1 μg base, respectively. After 3 min of infusion of either quinidine or saline at each rate the injections were repeated as in the order above.

Figure 1 illustrates the changes in perfusion pressure produced by the bolus injections of KCl, $CaCl_2$, $MgCl_2$, norepinephrine, and acetylcholine. At constant blood flow vasodilators such as KCl, $MgCl_2$, and

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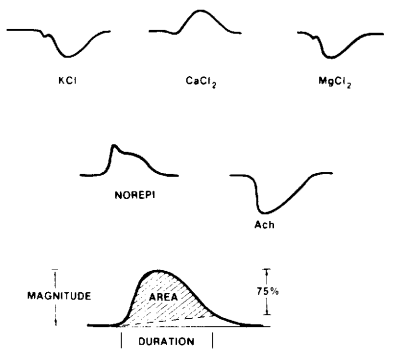

FIG. 1. Typical responses of perfusion pressure at constant flow to the vasoactive agents in the dog forelimb. The bottom schematic shows how the responses were quantitated.

acetylcholine decrease perfusion pressure while vasoconstrictors such as $CaCl_2$ and norepinephrine increase perfusion pressure. The response to norepinephrine includes both fast and slow phases. The lower trace in the figure illustrates how the changes in perfusion pressure were analyzed. The response magnitude represents the maximum change in perfusion pressure from the preinjection value in millimeters Hg. Constrictor responses were expressed as positive changes while dilator responses were expressed as negative changes. The area and duration of response were expressed in millimeters squared and millimeters, respectively, and were measured to the point at which the response had recovered by 75% to the preinjection level. The calibration of the recorder was such that 1 mm deflection represented a 5-mm Hg change in pressure and the paper advanced at 25 mm/minute.

Intergroup differences were statistically analyzed using Student's t test. Intragroup differences were evaluated using Student's t test modified for paired replicates. In the latter case, the values obtained during each infusion rate were compared to those obtained prior to infusion within the same group of animals. The level of significance was set at $P < 0.05$ as determined for a two-tailed test.

Results. The quinidine and saline infusions had no effect on perfusion pressure. They also failed to affect the magnitude, duration and area of the perfusion pressure responses to injections of KCl, $CaCl_2$, $MgCl_2$, and acetylcholine. Quinidine did however alter the response to norepinephrine. The slow phase of the response tended to disappear, resulting in a spike-like response. Average effects on magnitude, duration, and area are shown in Fig. 2. On an intragroup basis, quinidine treatment at all three levels reduced the duration and area of the response. Magnitude was slightly reduced only at the two highest levels of treatment. On an intergroup basis all three parameters were reduced at the two higher levels of treatment. None of the response parameters were affected by saline infusion.

Discussion. The vasoconstrictor pattern produced by a bolus injection of norepinephrine can be divided into two distinguishable phases (Fig. 1). The first phase is a fast increase in perfusion pressure which is relatively short lived. This component is referred to as the *fast phase* and is attributed to calcium derived from intracellular sources (11). The second phase of the norepinephrine response is more long lived than the fast phase. This component is referred to as the *slow phase,* thought to result from an inward flux of calcium across or from the sarcolemma (11). The magnitude of the response reflects the fast phase component while the duration and area of the response more closely reflect the slow phase component.

The lowest infusion rate of quinidine, which achieved a calculated plasma concentration of 6 μM (a level which falls below the 3–10 mg/liter plasma (9–31 μM) therapeutic range (1)), reduced the area and duration of the norepinephrine response without affecting the magnitude of the response. Thus, at this level, the effect was entirely on the slow phase of the norepinephrine response. The higher two infusion rates, 15 and 31 μM (which fall within the therapeutic range), also reduced magnitude somewhat. Qunidine has been previously shown to inhibit adrenergic vasoconstriction induced by either sympathetic nerve stimulation or by injection of norepinephrine but the mechanism of action was not defined (12, 13) (quinidine is

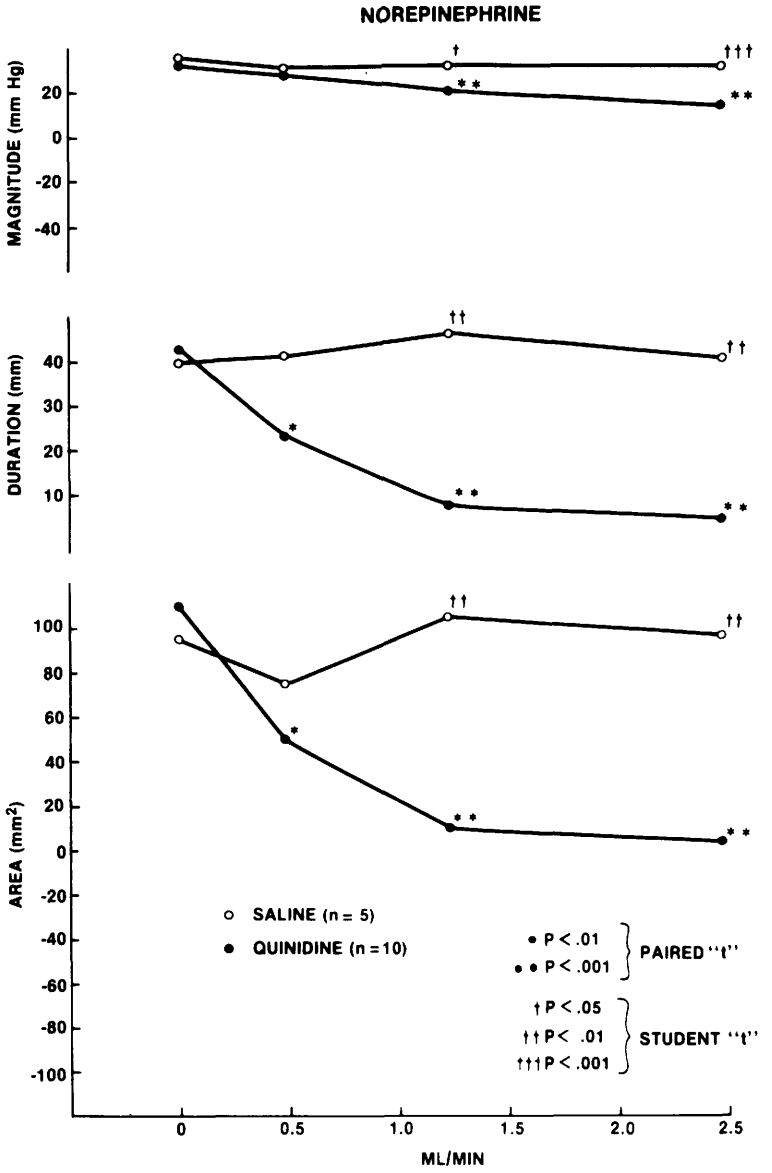

FIG. 2. Effects of saline and quinidine infusion on the responses to norepinephrine. The quinidine solution (quinidine hydrochloride) contained 500 μg/milliliter. The norepinephrine (levarterenol bitartrate) was administered as a 0.1-ml bolus which delivered 0.1 μg of the base. The stars indicate a significant difference relative to the control value in the same group. The crosses indicate a significant difference between groups at the same infusion rate.

also reported to have antiadrenergic activity in guinea pig atria (14)). Our study suggests that the inhibition results mainly from the blockade of an inward flux of calcium across or from the sarcolemma.

Quinidine had no effect of the responses to the cationic vasoactive agents, even though it inhibits microsomal Na^+, K^+-ATPase and Mg^{2+}-ATPase activities in a variety of nonvascular tissues (2–8) and even though ouabain is known to block potassium vasodilation (9, 10). En-

zymatic inhibition in microsomes prepared from mammalian tissue was however reported to occur at concentrations of quinidine ranging from 100 μM to 1 mM. These concentrations exceed those used in this study and may explain why we failed to see changes in the response to the cations.

Summary. Quinidine was shown not to affect the vascular response to KCl, $MgCl_2$, $CaCl_2$, or acetylcholine in the dog forelimb. Quinidine did greatly inhibit at therapeutic and nonvasodilating levels, the slow phase of the norepinephrine vasoconstrictor response. It is therefore concluded that the adrenergic inhibition of quinidine, reported here and by others, is mainly the result of an effect at the sarcolemma of the vascular smooth muscle cell which inhibits calcium influx and hence vasoconstriction.

1. Moe, G. K., and Abildskov, J. A., *in* "The Pharmacological Basis of Therapeutics" (L. S. Goodman and A. Gilman, eds.), p. 711. Macmillian Co., New York (1970).
2. Dhalla, N. S., Harrow, J. A. C., and Anand, M. B., Biochem. Pharmacol. **27**, 1281, (1978).
3. Kennedy, K. G., and Nayler, W. G., Biochim. Biophys. Acta **110**, 174 (1965).
4. Lowry, K. Rao, S. N., Pitts, B. J. R., and Askari, A., Biochem. Pharmacol. **22**, 1369 (1973).
5. Besch, H. R., and Watanabe, A. M., J. Pharmacol. Exp. Ther. **202**, 354 (1977).
6. Fuchs, F., Gertz, E. W., and Briggs, F. N., J. Gen. Physiol. **52**, 955 (1968).
7. Harrow, J. A. C., and Dhalla, N. S., Biochem. Pharmacol. **25**, 897 (1976).
8. Balzer, H., Naunyn-Schmiedeberg's Arch. Pharmacol. **274**, 256 (1972).
9. Chen, W. T., Brace, R. A., Scott, J. B., Anderson, D. K., and Haddy, F. J., Proc. Soc. Exp. Biol. Med. **140**, 820 (1972).
10. Bunger, R., Haddy, F. J., Querengasser, A., and Gerlach, E., Pflugers Arch. **363**, 27 (1976).
11. Healy, J. K., and Bohr, D. F., Proc. Soc. Exp. Biol. Med. **155**, 495 (1977).
12. Schmid, P. G., Nelson, L. D., March, A. L., Heistad, D. D., and Abboud, F. M., Pharmacol. Exp. Ther. **188**, 124 (1974).
13. Nelson, L. D., Schmid, P. G., Holmsten, D., Mark, A. L., Heistad, D. D., and Abboud, F. M., Proc. Soc. Exp. Biol. Med. **146**, 409 (1974).
14. Zetler, G., and Singbartl, G., Pharmacology **4**, 129 (1970).

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