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Cooling Augments Platelet-Induced Contraction of Peripheral Arteries of the Dog (41850)

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Abstract. The effect of cooling on platelet-induced contractions was studied. Rings of canine saphenous arteries were suspended for isometric tension recording in organ baths filled with aerated physiological salt solution. Norepinephrine, 5-hydroxytryptamine, and autologous aggregating platelets all caused contractions that were augmented by cooling the bath content from 37 to 24°C. These contractions were inhibited, in a concentration-dependent manner, by the serotonergic antagonist ketanserin. The alpha$_1$-adrenergic antagonist, prazosin, in concentrations causing progressive inhibition of contractions evoked by norepinephrine did not affect the response to either 5-hydroxytryptamine or platelets. Aggregating platelets were found to release 5-hydroxytryptamine in sufficient amounts to account for the observed contractions. Pretreatment of platelets with the cyclo-oxygenase inhibitor meclofenamate reduced the amount of thromboxane liberated by aggregating platelets but did not influence evoked contractions. These observations suggest that aggregating platelets cause contraction of the canine saphenous artery by releasing 5-hydroxytryptamine. They demonstrate that cooling markedly augments contractions of peripheral arterial smooth muscle caused by aggregating platelets.

Local cold enhances the constrictions of cutaneous vessels caused by norepinephrine released from adrenergic nerves or by circulating catecholamines; the action of other vasoactive agents, such as 5-hydroxytryptamine (serotonin) is also enhanced by cooling (1). Patients with Raynaud's phenomenon are characterized by prolonged constriction (spasm) of the digital arteries when exposed to a cold stimulation, particularly when they are emotionally upset. Platelets contain vasoconstrictor substances such as 5-hydroxytryptamine and thromboxane A_2. To judge from data obtained in isolated blood vessels, aggregating platelets can release these substances in amounts sufficient to cause sustained contraction of vascular smooth muscle (2, 3). Activation of platelets with subsequent release of vasoactive substances may be of pathogenic importance in patients with Raynaud's phenomenon (4–6). The present study was undertaken to determine the effect of local cold on platelet-induced contractions of peripheral arteries.

Materials and Methods. The experiments were performed on saphenous arteries taken from mongrel dogs of either sex (15–28 kg) anesthetized with sodium pentobarbital (30 mg/kg iv). The blood vessels were cut into rings (approximately 3 mm in length). The experiments were performed in organ chambers containing 25 ml of physiological salt solution (millimolar composition: NaCl, 118.3; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25.0; calcium-EDTA, 0.026; glucose, 11.1) and aerated with a 95% O_2–5% CO_2 gas mixture. Each ring was suspended by two stainless-steel stirrups passed through the lumen. One stirrup was anchored inside the organ chamber and the other was connected to a force transducer for recording of isometric tension (7, 8). All preparations were progressively stretched to the optimal length for contraction by measuring the contractile response to electrical stimulation of the adrenergic nerves (9 V, 2 msec, 16 Hz, 10 sec) (7, 9, 10). For cooling experiments, the temperature of water flowing through a water jacket surrounding the chamber was altered, thereby lowering bath temperature from 37 to 24°C in approximately 1.5 min (11). Bath temperature was continuously monitored using a thermistor probe.

Platelet suspensions were prepared from autologous blood collected in modified acid-citrate–dextrose anticoagulant (12). Because of the combined presence of Ca^{2+} in the physiological salt solution and collagen in the arterial ring, spontaneous platelet aggregation occurred following addition of 375 μl of the

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suspension to the bath containing the arteries as evidenced by clearing of the initially turbid solution and the release of 5-hydroxytryptamine and thromboxane B_2. In some experiments, platelet suspensions were incubated with meclofenamate (1×10^{-5} *M*) for 30 min and washed once before addition to the organ chamber.

5-Hydroxytryptamine determinations. Samples of physiological salt solution containing aggregated platelets were withdrawn from the organ chamber following stabilization of the vessel response to aggregating platelets. To determine the amount of 5-hydroxytryptamine released, 2-ml aliquots of bath fluid were centrifuged (3000*g*, 10 min, 4°C) and the supernatant was frozen at −20°C until analysis. Aliquots of supernatant were adjusted to pH 6.1 and applied to columns of cation exchange resin (Amberlite CG 50, 200–400 mesh). The amine was eluted and then separated by reversed-phase high-pressure liquid chromatography (μ Bondapak C_{18} column) with electrochemical detection (13).

Thromboxane B_2 determinations. Two-milliliter aliquots of thawed supernatant, obtained as above, were brought to pH 3.5 with 1 *M* citric acid. Thromboxane B_2 was extracted twice with chloroform:methanol (2:1, 6 ml), and the organic solvents were evaporated to dryness in a 37°C water bath under a stream of nitrogen. One-hundred-microliter aliquots of standards and diluted samples were assayed by displacement of [^{3}H]thromboxane B_2 from thromboxane B_2-antiserum in a total incubation volume of 300 μl at 4°C. The reaction was stopped after 2 hr by addition of a 1 ml solution of dextran-coated charcoal. After centrifugation (3000*g*, 5 min, 4°C), the supernatant containing the antibody-bound [^{3}H]thromboxane B_2 fraction was counted in a scintillation counter, and the concentration of thromboxane B_2 was estimated by comparison with a standard curve (14).

Drugs. The following pharmacological agents were used: 5-hydroxytryptamine creatinine sulfate, norepinephrine bitartrate (Sigma), ketanserin bitartrate (Janssen), prazosin hydrochloride (Pfizer), sodium meclofenamate (Parke-Davis), [^{3}H]thromboxane B_2 (New England Nuclear), and thromboxane B_2-antiserum (Seragen).

Prazosin was dissolved in 100 μl of dimethyl sulfoxide before addition to distilled water to make the stock solution. All other drugs were dissolved in distilled water. Concentration of drugs are expressed as final molar (*M*) bath concentration. Antagonists were added to the bath at least 20 min before determining their effect.

Calculations and statistical analysis. In each experimental group the number of rings equals the number of dogs. In all experiments involving repeated exposures to an agonist, control preparations obtained from the same vessel were studied in parallel to correct for changes in sensitivity with time.

When exposed to the agonists many of the rings showed a biphasic response—an initial peak followed by a smaller stable response. For calculations only the stable responses were used. The data are expressed as means ± SEM. Statistical analysis was performed using Student's *t* test for paired observations (two-tailed). Differences were considered to be significant if *P* values were less than 0.05.

Results. Platelets were added to the bath solutions (1.1×10^7 to 3.3×10^7/ml). Contractions of similar magnitude were obtained with aggregating platelets, exogenous 5-hydroxytryptamine (1×10^{-7} to 1×10^{-6} *M*) and norepinephrine (1×10^{-6} to 1×10^{-5} *M*) in control solution. The experiments were then repeated in the presence of increasing concentrations of prazosin and ketanserin (1×10^{-8} to 1×10^{-6} *M*). The contractions to norepinephrine were depressed in a concentration dependent fashion by both ketanserin and prazosin. Ketanserin but not prazosin caused a concentration-dependent depression of contractions to both platelets and 5-hydroxytryptamine (Fig. 1).

Following addition of platelet suspension ($1.9 \pm 0.6 \times 10^7$/ml, $N = 6$) to the organ chambers, completion of the platelet aggregation and stabilization of the vessel response the concentration of 5-hydroxytryptamine in the solution was $1.9 \pm 0.4 \times 10^{-7}$ *M*. Pretreatment of platelets with meclofenamate (1×10^{-5} *M*) for 30 min did not significantly influence contractions to aggregating platelets (5.3 ± 1.3 and 5.6 ± 1.5 g, with untreated and treated platelets, respectively; $N = 6$). The concentration of thromboxane B_2 was less in bath fluid withdrawn from organ chambers with meclofenamate incubated platelets than

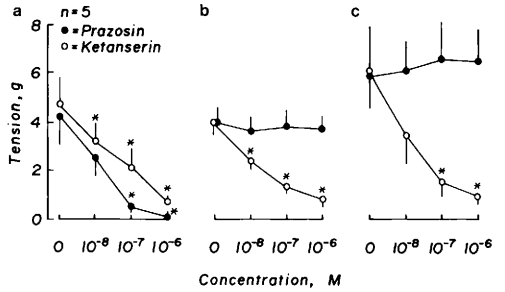

FIG. 1. Effect of increasing concentrations (1×10^{-8} to 1×10^{-6} *M*) of ketanserin and prazosin, respectively, on contractions evoked by (a) norepinephrine (1×10^{-6} to 1×10^{-5} *M*); (b) 5-hydroxytryptamine (1×10^{-7} to 1×10^{-6} *M*); and (c) aggregating platelets (1.1×10^7/ml to 3.3×10^7/ml). Asterisks denote a significant difference from control ($N = 5$).

in solution obtained from chambers where untreated platelets had been used ($4.5 \pm 1.3 \times 10^{-9}$ *M* and $1.2 \pm 0.3 \times 10^{-9}$ *M*, respectively; $N = 4$).

Cooling from 37 to 24°C had no significant effect on resting tension of the vessels ($N = 6$). Rings were contracted to comparable tension levels using exogenous norepinephrine (1×10^{-6} *M*), 5-hydroxytryptamine (1×10^{-7} to 3×10^{-7} *M*) and autologous aggregating platelets ($2.1 \pm 0.7 \times 10^7$/ml). After stabilization of the vessel response cooling from 37 to 24°C caused a similar augmentation of the contractions caused by norepinephrine, 5-hydroxytryptamine and aggregating platelets (Fig. 2). In rings ($N = 6$) contracted with 20 or 40 m*M* KCl, cooling caused significant decreases in tension (from 4.6 ± 0.8 to 1.7 ± 0.3 and 11.6 ± 1.8 to 5.3 ± 0.9 g, respectively).

Discussion. The present study confirms that when autologous platelets are added to physiological salt solution in organ chambers, the resulting aggregation causes the release of both 5-hydroxytryptamine and thromboxane (e.g., (2, 3)). The contraction of the canine saphenous artery to aggregating platelets is mediated mainly by 5-hydroxytryptamine. This conclusion is based on the observation that (a) inhibition of cyclo-oxygenase, causing a decreased release of thromboxane, did not affect the contractile response evoked by platelets; and (b) the serotonergic antagonist ketanserin, antagonized the platelet-induced contractions. Although the present study confirms that ketanserin also inhibits alpha$_1$-adrenoceptor activation (e.g., (15–18)), this is not likely to play a role in the antagonism of the platelet-evoked response, as the selective alpha$_1$-adrenergic antagonist prazosin was without effect.

As in other cutaneous vessels such as saphenous veins (see (1)) cooling greatly augmented the contractile response to exogenous norepinephrine and 5-hydroxytryptamine but reduces that to depolarizing solution (19, 20). Previous work in the saphenous vein of the dog has demonstrated that moderate cooling causes an instantaneous increase in the affinity of the postjunctional alpha-adrenoceptors for norepinephrine (11). The comparison between the effect of cooling on responses to norepinephrine and 5-hydroxytryptamine suggests that cooling affects the response to both monoamines in a comparable fashion in the saphenous artery. The present study demonstrates that, despite the possible inhibitory effect that cooling may have on the aggregation-release reaction (21), the increased responsiveness of the smooth muscle cells allows for a markedly augmented response to platelets at the lower temperature tested.

Activation of platelets has been reported in patients with peripheral vascular disease characterized by cold-induced vasospasm. These conditions include Raynaud's disease (4–6) as

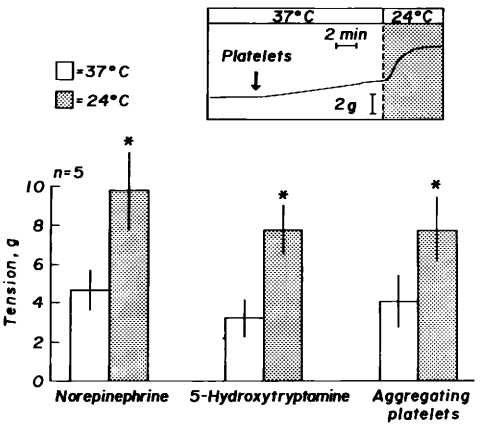

FIG. 2. Contractile tension at 37 and 24°C evoked by (a) norepinephrine (1×10^{-6} *M*); (b) 5-hydroxytryptamine (1×10^{-7} to 3×10^{-7} *M*); and (c) aggregating platelets ($2.1 \pm 0.7 \times 10^7$/ml). Asterisks denote significant difference between values and 37 and 24°C ($N = 5$).

well as Raynaud's phenomenon induced by use of vibrating tools (4). The present observations on canine cutaneous arteries suggest that the cold-induced augmentation of the contractile response to aggregating platelets, mediated by released 5-hydroxytryptamine may be of importance *in vivo*. This would help explain the favorable therapeutic effect of ketanserin in patients with Raynaud's phenomenon (22).

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1. Vanhoutte PM. Physical factors of regulation. In: Bohr DF, *et al*, eds. Handbook of Physiology. Baltimore, Williams & Wilkins, Sec 2, Vol 2:p447, 1980.
2. De Clerck F, Van Nueten JM. Platelet-mediated vascular contractions: Inhibition of the serotonergic component by ketanserin. Thromb Res **27**:713–727, 1982.
3. Cohen RA, Shepherd JT, Vanhoutte PM. Inhibitory role of the endothelium in the response of isolated coronary arteries to platelets. Science **221**:273–274, 1983.
4. Blunt RJ, George AJ, Hurlow RA, Strachan CJL, Stuart J. Hyperviscosity and thrombotic changes in idiopathic and secondary Raynaud's syndrome. Brit J Haematol **45**:651–658, 1980.
5. Zahavi J, Hamilton WAP, O'Reilly MJG, Leyton J, Cotton LT, Kakkar VV. Plasma exchange and platelet function in Raynaud's phenomenon. Thromb Res **19**:85–93, 1980.
6. Kallenberg CGM, Vellenga E, Wouda AA, Hauwthe T. Platelet activation, fibrinolytic activity and circulating immune complexes in Raynaud's phenomenon. J Rheumatol **9**:878–884, 1982.
7. De Mey J, Vanhoutte PM. Interaction between Na^+,K^+ exchanges and the direct inhibitory effect of acetylcholine on canine femoral arteries. Circ Res **46**:826–836, 1980.
8. De Mey J, Vanhoutte PM. Uneven distribution of postjunctional $alpha_1$ and $alpha_2$-like adrenoceptors in canine arterial and venous smooth muscle. Circ Res **48**:875–884, 1981.
9. Vanhoutte P, Clement D, Leusen I. The reactivity of isolated veins to electrical stimulation. Arch Int Physiol Biochem **75**:641–657, 1967.
10. Vanhoutte P, Leusen I. The reactivity of isolated venous preparations to electric stimulation. Pfluegers Arch **306**:341–353, 1969.
11. Janssens WJ, Vanhoutte PM. Instantaneous changes of alpha-adrenoceptor affinity caused by moderate cooling in canine cutaneous veins. Amer J Physiol **234**:H330–H337, 1978.
12. Dewanjee M, Rao SA, Didisheim P. Indium-111 tropolone, a new high-affinity platelet label: Preparation and evaluation of labelling parameters. J Nucl Med **22**:981–987, 1981.
13. Tyce GM, Yaksh TL. Monoamine release from cat spinal cord by somatic stimuli: an intrinsic modulatory system. J Physiol **314**:513–529, 1981.
14. Strand JC, Edwards BS, Anderson ME, Romero JC, Knox FG. Effect of imidazole on renal function in unilateral ureteral obstructed rat kidneys. Amer J Physiol **240**:F508–F514, 1981.
15. Van Nueten JM, Janssen PA, Van Beek J, Xhoneux R, Verbeuren TJ, Vanhoutte PM. Vascular effects of ketanserin (R 41 468), a novel antagonist of 5-HT_2 serotonergic receptors. J Pharmacol Exp Ther **218**:217–230, 1981.
16. Van Nueten JM, Leysen JE, Schuurkes JAJ, Vanhoutte PM. Ketanserin: A selective antagonist of 5-HT_2 serotoninergic receptors. Lancet Feb 5, 297–298, 1983.
17. Fozard JR. Mechanism of the hypotensive effect of ketanserin. J Cardiovasc Pharmacol **4**:829–838, 1982.
18. Kalkman HO, Timmermans PBWM, van Zwieten PA. Characterization of the antihypertensive properties of ketanserin (R41468) in rats. J Pharmacol Exp Ther **222**:227–231, 1982.
19. Vanhoutte PM, Shepherd JT. Effect of temperature on reactivity of isolated cutaneous veins of the dog. Amer J Physiol **218**:187–190, 1970.
20. Rusch NJ, Shepherd JT, Vanhoutte PM. The effect of profound cooling on adrenergic neurotransmission in canine cutaneous veins. J Physiol (London) **311**:57–65, 1981.
21. Sixma JJ. Methods for platelet aggregation. In: Triplett DA, ed. Platelet Function, Laboratory Evaluation and Clinical Application. Chicago, Amer Soc Clin Pathol, p87, 1978.
22. Stranden E, Roald OK, Krogh U. Treatment of Raynaud's phenomenon with the 5-HT_2 receptor antagonist ketanserin. Brit Med J **285**:1069–1071, 1982.

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