Inhibition of Constriction-Induced Reactive Hyperemic Edema (36198)

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Reactive hyperemia is a phenomenon characterized by an increase in blood flow to a tissue after temporary deprivation of its blood supply. Bayliss (1) attributed this excess flow to relaxation of the muscular coat of arterial walls subsequent to decreased blood pressure. Subjecting the muscle of an artery to reduced pressure may also lead to atrophy of its media (2). When such media is suddenly exposed to an elevated blood pressure as occurs after constriction, it may become overstretched, and may either fragment or rupture. If this occurs near the microvasculature, transudation of capillary fluid, including plasma proteins, into interstitial spaces could then ensue. Hyperemia precedes or accompanies edema of the foot and leg following arterial reconstruction, a procedure involving arterial constriction (2). Such edema presents a clinical problem and may be a manifestation of the phenomena just described. The present study describes a new method of inducing constriction-induced reactive hyperemic edema (RHE) in rats and the influence of vasoconstrictors and other drugs on RHE. Results suggest that certain drugs may be useful in treating RHE clinically.

Methods. Constriction-induced hyperemic edema. Edema in rat (male, Holtzman, 140–240 g) hind paws was induced by 13 to 18 turns of rubber bands (ca. 22 mm diam; 1.5 mm wide wide by 1.0 mm thick) applied as tightly as possible to the ankle joints of the right hind paws. This procedure usually required 30 to 50 sec; the band was removed with scissors. Left control paws were not constricted. Edema, determined plethysmographically by measuring the volume of mercury displaced (3, 4), was measured by subtracting the volume of the control, left hind paw from that of the swollen, right hind paw. This is *not* tourniquet-induced shock; no animals died when observed for at least 1 week. Moreover, edema completely disappeared within 2 to 4 days. Inhibition of edema was calculated by comparing the edema of control and drug-treated rats 1 hr after the constrictor band was removed.

Histamine edema. Histamine edema was induced in male rats (Harlan, Wistar, 140–200 g), by injecting histamine (300 μg of base) in 0.1 ml of 0.9% saline into the subplantar region of the right hind paw; saline (0.1 ml) was injected into the left. Effective inhibitory dose values (EID) were obtained as previously described (3) by plotting log dosage vs. percentage inhibition of edema using three to five dose levels and 5 or 10 rats per dosage; EID values indicate dose levels that inhibit edemic responses by 50% when compared to responses obtained in vehicle-treated animals.

Test drugs. The following drugs were included in this study: alphaprodine HCl, atropine sulfate, butylated hydroxyanisole (Nutritional Biochemicals Corp.), carboxypeptidase B (Sigma Chemical Co.; crystalline), cyproheptadine HCl, dexamethasone, l-epinephrine bitartrate, hexamethonium chloride dihydrate, histamine diphosphate, hydrocortisone, indomethacin, l-isoproterenol bitartrate dihydrate, meperidine HCl, methdilazine HCl, methysergide maleate, morphine sulfate, l-norepinephrine bitartrate monohydrate, phenylbutazone, phenylephrine HCl, phentolamine methanesulfonate, sotalol, soybean trypsin inhibitor (Sigma Chemical Co.) and tripelennamine HCl. Drug dosage is expressed as the weight of the salt. Fresh solutions or suspensions[1] were prepared daily in 10% (v/v) polyoxyethylated vegetable oil (Emulphor

[1] When suspensions were injected, which was not often, the sites of injection of the drug were inspected for unabsorbed drug particles; none were found.

TABLE I. Effect of Duration of Constriction on the Volume of Reactive Hyperemic Edema Induced.

Constriction (min)	Reactive hyperemic edema (ml ± SE); after constriction removed: (hr): 1	3
2.0	0.58 ± 0.05	0.43 ± 0.05
5.0	0.44 ± 0.04	0.31 ± 0.03
15.0[a]	0.44 ± 0.03	0.30 ± 0.02
15.0	0.47 ± 0.05	—
15.0	0.44 ± 0.03[b]	0.36 ± 0.04[b]
15.0	0.44 ± 0.06[c]	0.36 ± 0.03[c]
30.0	0.40 ± 0.04	—
30.0	0.52 ± 0.06	0.38 ± 0.04
60.0	0.72 ± 0.03[b]	0.57 ± 0.05[b]

[a] Inhibition of RHE (15 min constriction) by drugs in Tables II–IV was calculated by comparing the mean edema (ml ± SE) observed in drug-treated animals to the mean edema value (0.45 ± 0.02, $n = 40$) 1 hr after constriction compiled from the control data listed above.

[b] Animals were pretreated 30 min prior to constriction by drug vehicle (polyoxyethylated vegetable oil, 2 ml/kg, sc) injected between the scapulae.

[c] Pretreated with drug vehicle (2 ml/kg, sc) 60 min, and again 30 min, prior to constriction.

EL-620, General Aniline Co.) and usually injected subcutaneously (2.0 ml/kg) between the scapulae 30 min prior to constriction.

Results. Duration of constriction. Edema occurred simultaneously with hyperemia; the latter was indicated by the extremely large degree of redness of the previously constricted, cyanotic right hind paws. The relative standard error of edema varied from 4 to 14% based on all of the control values listed in Table I. Data in Table I also indicate little effect of duration of constriction on the volume of edema induced at either the 1 or 3 hr postconstriction interval. Edema after 60 min of constriction was, however, significantly greater. Administration of drug vehicle once, or twice, prior to constriction had no significant effect on the degree of swelling. Constriction of less than 2 min was not feasible since the constriction procedure required 30 to 50 sec. Pain, which seemed quite severe, was manifested by all control animals during constriction and after constriction for as long as 60 min; this nociceptive reaction was always accompanied by limping, marked aggressiveness, and vocalization.

Effect of drugs. Atropine at rather high dosage had no significant inhibitory effect on RHE (Table II). Inhibition of RHE was affected by phenylephrine, a potent and rather pure α-sympathomimetic vasoconstrictor (Table II). Similar results were obtained with norepinephrine but at smaller dosages. A lesser degree of attenuation was induced by the β-sympathomimetic isoproterenol. Neither sotalol, a potent β-adrenergic receptor blocking agent, nor phentolamine, a potent α-adrenergic receptor blocking agent, antagonized its inhibitory effect (Table III). The inhibitory effects of both norepinephrine and epinephrine (Table III) were blocked, however, by phentolamine, but not by sotalol. Ganglionic blockade induced by high dosage of hexamethonium did not attenuate RHE (Table II). Phentolamine, at dosage higher than that required to

TABLE II. Modification of Reactive Hyperemic Edema by Atropine, Sympathomimetic and Sympatholytic Drugs.

Compound	Dose[a]	Inhibition (%) of edema
Atropine	75.0	13
Epinephrine	5.0	98[b]
	2.5	87[b]
	1.0	51[b]
	0.25	36[b]
Hexamethonium	100.00	9
Isoproterenol	20.0	36[b]
	10.0	49[b]
	5.0	22[b]
Norepinephrine	2.5	71[b]
	1.0	53[b]
	0.5	42[b]
Phenylephrine	18.0	96[b]
	6.0	80[b]
	2.0	24[b]
Phentolamine	20.0	60[b]
	2.0	18

[a] In mg/kg, sc; drugs were administered between the scapulae 30 min prior to constriction. Constriction in this and all other drug studies was of 15 min duration.

[b] $p < .05$ compared with vehicle-treated controls (see Table I); $n = 8$ to 10 in drug-treated groups.

TABLE III. Effect of Adrenergic Receptor Blockade on Inhibition of Reactive Hyperemic Edema by Sympathomimetics.

Pretreatment[a]	Treatment	Inhibition (%) of edema
Vehicle (2 ml/kg)	Epinephrine (2.5)	87[b]
	Epinephrine (1.0)	51[b]
Sotalol (10.0)	Epinephrine (2.5)	91[b]
Phentolamine (2.0)	Epinephrine (1.0)	22[b,c]
None	Isoproterenol (10.0)	49[b]
Sotalol (50.0)[a]	Isoproterenol (10.0)	53[b]
Phentolamine (2.0)	Isoproterenol (10.0)	56[b]
Vehicle (2 ml/kg)	Norepinephrine (1.0)	53[b]
Phentolamine (2.0)	Norepinephrine (1.0)	13[b]
Sotalol (10.0)	Norepinephrine (1.0)	73[b,c]
None	Sotalol (50.0)	13
Sotalol (10.0)	Vehicle (2 ml/kg)	18
Phentolamine (2.0)	Vehicle (2 ml/kg)	18

[a] Drug pretreatment 60 min prior to constriction; drug treatment 30 min prior to constriction, except in the case of sotalol (50.0) and isoproterenol (10.0) which were administered simultaneously 30 min prior to constriction. Values in parentheses indicate dosage (mg/kg sc).

[b] $p < .05$ compared to vehicle-treated, control animals; $n = 10$ in drug-treated groups.

[c] $p < .05$ compared to epinephrine or norepinephrine alone at the same dosage.

antagonize the inhibitory effects of the α-sympathomimetics, also inhibited RHE (Table II).

Methysergide maleate, a potent antiserotonin drug, had only a slight inhibitory effect. In serotonin edema, 0.05 mg/kg (po of methysergide maleate given 30 min prior to the subplantar injection of 10 μg of serotonin base (edema determined 1 hr after serotonin) reduced the edema by 50%. In RHE, 1000 times that amount of methysergide maleate (50 mg/kg, sc) yielded only 27% inhibition of RHE. Moreover, the subcutaneous dosage (shown in parentheses) of methdilazine (6.0 mg/kg) that reduced serotonin (10 μg)-induced edema by 50% (6), is much less (ca. 10-fold than the dosage (ca. 75 mg/kg) needed to cause a 50% reduction in RHE. Methdilazine and tripelennamine, potent antihistamines (4,6), inhibited RHE only at high dosage. The ED value for methdilazine in histamine edema (1.6 mg/kg, po) is much less than 75 mg/kg. Indeed, cyproheptadine, a potent antiserotonin/antihistaminic drug (6), did not inhibit RHE but inhibited histamine edema at a dosage of 1.0 mg/kg, po.

Carboxypeptidase B (0.5 mg in 0.1 ml of saline/paw in the subplantar region 5 min prior to constriction), which destroys bradykinin, and soybean trypsin inhibitor (2 mg/paw, as above), which inhibits trypsin, and bradykinin formation, had no inhibitory effect on RHE in 10 rats. However, the antibradykinin agent, butylated hydroxyanisole (7), had a slight inhibitory effect (38%) on RHE at high dosage (400 mg/kg, sc). It also caused a similar degree of inhibition (36%) of edema induced 30 min after the subplantar injection of bradykinin triacetate (25 μg/paw in 0.1 ml of saline) in 10 rats.

Steroidal anti-inflammatory drugs (hydrocortisone and dexamethasone, 40 mg/kg, sc) were ineffective in attenuating RHE. Indomethacin and phenylbutazone, however, caused a slight reduction (20 to 31%) of RHE at high dosage (40 to 65 mg/kg, sc). Thus, classical anti-inflammatory agents have little or no effect on RHE.

The role of the CNS in attenuating the development of edema in rat hind paws is again (3) demonstrated by the significant inhibition of RHE by narcotic "analgesic/anti-inflammatory" drugs (Table IV). Their intrinsic activity, however, is about half that of the α-sympathomimetric drugs since increasing dosages of morphine resulted in no

TABLE IV. Inhibition of Reactive Hyperemic Edema by Narcotic Analgesic/Anti-inflammatory Drugs.

Compound	Dose[a]	Inhibition (%) of edema
Alphaprodine	9.0	49[b]
	3.0	24[b]
	1.0	20
Meperidine	80.0	49[b]
	60.0	40[b]
	40.0	24[b]
Morphine	20.0	42[b]
	10.0	40[b]
	5.0	44[b]
	2.5	13

[a] In mg/kg, sc, injected between the scapulae 5 min prior to constriction.

[b] $p<.05$ compared with vehicle-treated controls; $n = 10$ in drug-treated groups.

more than 42% inhibition of RHE. Phentolamine (2.0 mg/kg) did not antagonize the attenuating effect of alphaprodine (9.0 mg/kg), when given 30 min prior to the narcotic.

Reserpine (5.0 mg/kg, ip), when given 20 hr prior to constriction in 10 rats, caused 60% inhibition of RHE. In another experiment involving 10 rats, the same dosage of reserpine given 20 hr earlier neither inhibited nor potentiated morphine (5.0 mg/kg), *i.e.*, 47% inhibition of RHE was observed.

Discussion. The role of the cholinergic nervous system in the development of RHE is probably of no importance since atropine at rather high dosage had no inhibitory effect. That overstretching of precapillary smooth muscle occurs after constriction is terminated is indicated by the pronounced inhibition caused by α-sympathomimetics. The latter presumably act on the arteriolar side of the microvasculature by stimulating α-adrenergic receptors since constriction of the venular side would probably either augment RHE or have no effect. In addition, phentolamine, but not sotalol, antagonized their inhibitory effects. These results support the theory of Bayliss (1). The mechanism by which phentolamine per se depresses RHE is unknown, but since it produces vasodilation in skin and muscle (5) this mechanism cannot be invoked. A CNS-mediated effect cannot be ruled out, however, since symptoms of marked ptosis and moderate depression accompanied its inhibitory action. Similar symptoms were noted with reserpine, which also inhibited RHE.

An immediate and/or continued release of histamine is probably not responsible for the development of RHE, since cyproheptadine, a potent antiserotonin/antihistamine, did not inhibit RHE. Methdilazine did inhibit RHE but only at high dosage compared to that required to inhibit histamine edema in rat hind paws. Moreover, histamine induces a lesser degree of edema in rat hind paws after it is injected repeatedly in amounts up to 300 μg (6). In addition, histamine edema is fleeting; whereas RHE is persistent. Methysergide given parenterally yields less inhibition of RHE than serotonin edema in rat hind paws at 1000 times the dosage required to inhibit serotonin edema by 50%. Thus it is concluded that serotonin likewise plays no important role in the development of RHE. Bradykinin may be involved in the onset of RHE since butylated hydroxyanisole, an antibradykinin agent (7), caused a similar degree of inhibition (38%) of RHE and edema induced by bradykinin triacetate (25 μg/paw in 0.1 ml of saline), viz. 36% inhibition. However, carboxypeptidase B (0.5 mg in 0.1 ml of saline/paw in the subplantar region 5 min prior to constriction), which destroys bradykinin, and soybean trypsin (2 mg/paw), which inhibits trypsin, and bradykinin formation, had no inhibitory effect on RHE. In contrast, both of these agents in similar, or identical, amounts inhibit carrageenin edema (8, 9).

Results with reserpine and possibly phentolamine and the narcotics suggest that depletion of brain dopamine or norepinephrine levels may be involved in the inhibition of RHE. Morphine does lower the levels of both dopamine and norepinephrine in specific regions of the CNS including the hypothalamus (10, 11). The catecholamine-depleting action of reserpine in rat brain, at the dosage used in the present study, is well known. Catecholamine release (and possibly depletion) is also well established for phentolamine (12). The inhibitory effects of the narcotics also suggest that compression or chemical stimulation of sensory nerves during or after constriction, re-

spectively, is followed by nerve transmission to supraspinal centers. Since pathways between the CNS and hind paws are needed to obtain the early phase of acute edemic responses (3), narcotics may inhibit RHE by a mechanism similar to that induced by cutting the spinal cord or peripheral nerves leading to the hind paw (3).

Summary. Reactive hyperemic edema (RHE) was induced in hind paws of rats by application of a constriction band for 2, 5, 15, 30, or 60 min; 1 or 3 hr after removal of the band, swelling of the hind paws was measured. RHE was completely reversible within 2 to 4 days; none of the animals died. Attenuation of RHE was produced by the vasoconstrictor, α-sympathomimetic activity of norepinephrine, phenylephrine, or epinephrine; phentolamine antagonized this attenuation completely, but sotalol, a β-adrenergic receptor blocking agent, did not. Tripelennamine and methdilazine produced slight inhibition of RHE at much higher dose levels than those needed to reduce histamine-induced edema in hind paws; cyproheptadine (50 mg/kg, sc) was ineffective. Methysergide maleate (50.0 mg/kg) caused only a slight reduction of RHE (27%). Classical anti-inflammatory steroids were ineffective but indomethacin and phenylbutazone induced a small degree of reduction of RHE at high dosage. Narcotic analgesic/anti-inflammatory drugs, morphine, meperidine, and α-prodine, inhibited RHE according to their rank order of analgesic potency but none produced more than 49% inhibition. Atropine (75 mg/kg) and hexamethonium (100 mg/kg) did not diminish RHE. It is concluded that RHE may be induced by overstretched, fragile, smooth muscle of precapillary vessels; and constriction of the latter by an α-sympathomimetic action, either directly or indirectly (by affecting certain regions of the central nervous system which may be concerned with the peripheral release of α-sympathomimetics), inhibits the development of RHE. Histamine or serotonin are probably not involved in the development of RHE.

1. Bayliss, W. M., J. Physiol. (London) **28**, 220 (1902).

2. Husni, E. A., Circulation **35** (Suppl.), 169 (1967).

3. Brown, J. H., Kissel, J. W., and Lish, P. M., J. Pharmacol. Exp. Ther. **160**, 231 (1968).

4. Lish, P. M., Albert, J. R., Peters, E. L., and Allen, L. E., Arch. Int. Pharmacodyn. Therap. **129**, 77 (1960).

5. Johnson, H. D., Green, H. D., and Lanier, J. T., J. Pharmacol. Exp. Ther. **108**, 144 (1953).

6. Brown, J. H., Mackey, H. K., Riggilo, D. A., and Schwartz, N. L., J. Pharmacol. Exp. Ther. **160**, 242 (1968).

7. Posati, L. P., and Pallansch, M. J., Science **168**, 121 (1970).

8. Van Arman, C. G., Begany, A. J., Miller, L. M., and Pless, H. H., J. Pharmacol. Exp. Ther. **150**, 328 (1965).

9. Van Arman, C. G., Nuss, G. W., Winter, C. A., and Flataker, L., *in* "Pharmacology of Pain" (R. K. S. Lim, D. Armstrong, and E. F. Pardo, eds.), p. 25. Pergamon, Elmsford, NY (1966).

10. Dole, V. P., Ann. Rev. Biochem. **39**, 821 (1970).

11. Clouet, D. H., and Ratner, M., Science **168**, 854 (1970).

12. Nickerson, M., *in* "The Pharmacological Basis of Therapeutics" (L. S. Goodman and A. Gilman, eds.), 4th ed. p. 560. Macmillan Co., New York (1970).

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