size of the lesions varied from small to complete VMH ablation. The low incidence of lesions in Group 3 may be related to an increased ability to metabolize the lactate due to training. In the Figure are photomicrographs of the VMH of the brain of diabetic mice, one exercized and the other not exercized. It should be noted that none of the unexercized mice (Group 1) developed VMH lesions following GTG injection, while 15 of 24 normal mice given doses of only 0.3 mg/g body weight developed lesions.

In a second experiment untrained diabetic mice (Group 6) were injected i.p. with 2.5 μmoles/g of Na lactate (pH = 7.34) and 30 min later with 0.8 mg/g GTG. Groups 7, 8, 9 and 10 were trained as described above and on the day following the training period were first injected with 2.5 μmoles/g of Na lactate and then 0.8 mg/g GTG 0.5, 2, 4 and 8 h later, respectively. These mice also developed lesions in the VMH, even up to 8 h after lactate injection (Table). Sodium propionate, 2.5 μmoles/g (pH = 7.34), was not effective in mediating the GTG lesions of the VMH of diabetic mice.

The results of the above experiment are evidence that the metabolism of the VMH of the diabetic mice following exercize or injection of lactate was changed in a manner similar to that of a diabetic mouse given insulin. Although we have no evidence that there is a relationship to these experiments, it is interesting that a factor released by muscle during exercize has insulin-like activity on glucose metabolism on various tissues including the brain (8, 9, 10). It is especially relevant that such a factor has been found in the urine of trained rats up to 12 h after the session of exercize[8]. It may be that this factor or one with similar properties is released by increased plasma levels of lactate and has insulin-like activity on the VMH which increases the metabolic and presumably the firing rate. Since it is a long-acting factor, it may cause a sustained hypophagia through the suppression of the lateral hypothalamic area activity by the increased VMH activity.

The importance of exercize in the treatment of diabetes has long been stressed[11]. Our experiments suggest that, in addition to the improvement of glucose utilization generally observed, exercize may also be beneficial in controlling the development of obesity not only by increasing energy expenditure but also by preventing excessive food intake[12].

Zusammenfassung. Erhöhte Lactatmengen im Plasma können während körperlicher Anstrengung die Freisetzung eines Stoffes verursachen, welcher, ähnlich wie Insulin, im mittleren Hypothalamus wirkt. Dieser Stoff könnte auch an der Auslösung der Hypophagie, welche nach körperlicher Anstrengung auftritt, beteiligt sein.

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The effect of exercize or lactate on the lesioning of the ventromedial hypothalamus by goldthioglucose (GTG) in mice made diabetic with alloxan

Group	*n*	Blood glucose (mg/100 ml)[a]	Condition	GTG injection time After exercize (min)	After lactate[b] (min)	Lesions (%)
1	9	398 ± 91	U[c]	N[e]		0
2	5	514 ± 33	U	0		60
3	11	477 ± 37	T[d]	0		18
4	5	534 ± 98	T	120		60
5	5	531 ± 27	T	240		0
6	5	411 ± 50	U		30	40
7	5	487 ± 55	T		30	40
8	5	437 ± 55	T		120	40
9	5	397 ± 20	T		240	80
10	5	558 ± 128	T		480	40

[a] Day of injection. [b] 2.5 μmoles/g body weight. [c] Untrained. [d] Trained. [e] Not exercized or injected with lactate.

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Physiologic and Pharmacologic Responses of Mammalian Vascular Smooth Muscles During Electric Field Stimulation

Previous studies[1-4] have shown that brief pulses of 60 Hz alternating current field stimulation (AC) cause contraction of various types of isolated smooth muscle. A portion of the response in vascular smooth muscle appears to be due to the release of endogenous catecholamines, principally norepinephrine[1,5]. We will demonstrate that essentially steady-state levels of contraction can be achieved during continuous AC stimulation, and that voltage-response curves can thus be generated. These curves are depressed by 'direct' smooth muscle relaxants, by an alpha-adrenergic blocking agent, and by ultra-violet radiation, which is known to reduce active tone[6]. Evidence will be presented that the non-catecholamine mediated portion of the AC response is due to stimulation of the excitable membrane, and perhaps to direct activation of the excitation-contraction coupling mechanisms.

Materials and methods. Spiral strips of aorta from rabbits killed by cervical concussion were suspended under a tension of approximately 3 g in a constant temperature bath, according to established methodology[7]. The bathing medium was Krebs bicarbonate solution

which contained 10^{-5} g/ml disodium ethylenediamine tetraacetate (versene) and was gassed with 95% O_2 – 5% CO_2, and maintained at 37 ± 0.5 °C. Rabbits pretreated with reserpine (Serpasil, CIBA), 2.5 mg/kg were dosed i.v. and used after 4 or 24 h. Dog femoral arterial strips for blood superfusion experiments[8] were taken from pentobarbital-anesthetized animals and were prepared as above. All spiral muscle strips were cut to 1 cm lengths, and were suspended in the bath between 2 platinum wire electrodes for AC stimulation. Field strength was regulated by a rheostat, and was monitored with a voltmeter. For superfusion, dog femoral arterial strips arranged as above were bathed in continuously flowing blood channeled from a carotid artery of the donor dog; a pulsatile pump returned the blood to the animal via a jugular vein. Drugs were injected into a femoral vein.

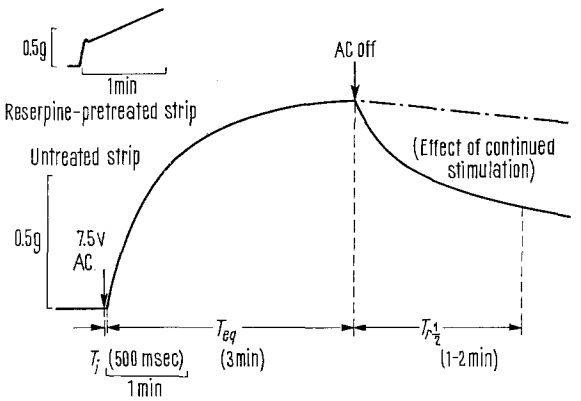

Fig. 1. Effect of prolonged 60 Hz AC field stimulation on rabbit aortic strips from a normal and from a reserpine pretreated rabbit (inset). Ti, time between beginning of stimulus and onset of contraction; Teq, time to equilibrium after beginning of stimulus; Tr 1/2, half time for relaxation after cessation of stimulus. Isometric recording.

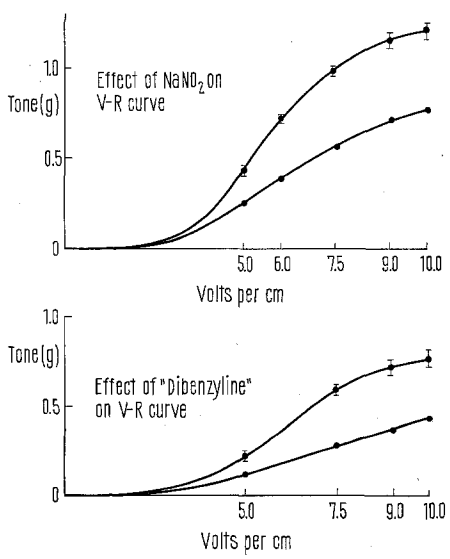

Fig. 2. Effect of $NaNO_2$, 2×10^{-4} g/ml (upper panel) and of phenoxybenzamine, 10^{-5} g/ml ('Dibenzyline' lower panel) on voltage response curves. Bars represent standard errors of the mean of three successive controls.

Results and discussion. Rabbit aortic strips exhibited smooth tonic contractions of 0.25–2 g when stimulated continuously with a moderate AC voltage (Figure 1). Prolonged stimulation was accompanied by gradual loss of tone, whereas cessation of AC resulted in a more rapid relaxation with a half-time of approximately 1–2 min. Responses could be reproduced with minimal intervals of 1 h between stimulations. Almost all the strips taken after 24 h from reserpine-pretreated rabbits (92% of a 30 strip sample) exhibited multiphasic contractions (inset, Figure 1).

Voltage-response curves and single responses were depressed by α-adrenergic blockade with phenoxybenzamine (10^{-5} g/ml), by 'direct' smooth muscle relaxing agents such as $NaNO_2$ (2×10^{-4} g/ml) (Figure 2) or hydralazine (10^{-4} g/ml), and by UV-radiation. Furthermore, $NaNO_2$ and UV-radiation reduced the AC tone in tissues pretreated for 20 min with phenoxybenzamine (10^{-5} g/ml). Strips from rabbits reserpinized 4 h before the experiment gave AC responses reduced in magnitude to those seen in normal strips but were not further reduced by phenoxybenzamine. It appeared that a portion of the AC response in normal tissues was due to the release of endogenous catecholamines, presumably norepinephrine; based on the phenoxybenzamine reduction of AC tone, this portion is estimated at 20–40%.

Strips that were maximally contracted by AC were contracted further by high doses of epinephrine (10^{-4} g/ml), and the converse was also true. This indicates that the 2 agents work, at least in part, at different sites, AC acting distal to the α-receptor. In a similar way, tissues depolarized with K_2SO_4-Krebs solution[2-9] were contracted further with AC, and the converse was true. It appears, then, that the AC response is due in part to activity beyond the excitable membrane, perhaps at the excitation-contraction coupling step. Efforts to block the latter with tetracaine were unsuccessful, since the agent caused contracture at doses ($10^{-3}M$) known to block calcium movement[10].

The tone induced by AC can be maintained in a continuously flowing medium. Dog femoral arterial strips exhibited the same responses as those of the rabbit aorta. The former when contracted under continuous AC stimulation showed a reduction of tone when superfused continuously with the blood of a donor dog injected intravenously with a known vasodilator, even after pretreatment of the strips with phenoxybenzamine.

It appears from this study that the contractile action of AC is via the excitable membrane, and perhaps at some step beyond. The antagonism of AC-induced tone by 'direct' smooth muscle relaxants may be interpreted as an indication that these agents (and UV-radiation) act at the same sites. These conclusions are in agreement with the work of other investigators[2,10] who used non-vascular smooth muscle. AC stimulation of vascular

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smooth muscle provides a unique method for determining the action of suspected vasodilating agents. The method has particular merit for evaluating agents with limited aqueous solubility.

Résumé. Des sections de muscle lisse d'aorte de lapin ou d'artère fémorale de chien répondent à une stimulation de champ (AC) par une contraction due en partie à la libération de catécholamines, contraction pouvant être relâchée sous l'effet de divers médicaments. D'autre part, l'AC manifeste une action au niveau de la membrane excitable et au-delà de cette membrane. Il en a peut-être aussi sur le couplage excitation-contraction. L'AC offre une méthode unique pour l'étude des agents susceptibles d'avoir une action vaso-dilatatrice.

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An Assay Procedure for Mescaline and its Determination in Rat Brain, Liver and Plasma

Trimethoxyphenylethylamine or mescaline is a known hallucinogenic compound in man[1] and produces abnormal behavior in animals[2]. Recently the behavioral effects of mescaline were compared with those obtained after the administration of similar methoxylated phenylethylamines[3,4]. Since the concentrations of most of these compounds in the CNS are unknown these structure-activity relationships had to be based on quantities injected. In order to measure the concentrations of mescaline in the CNS after injection of the compound, a rapid assay procedure was developed and the brain, liver, and plasma levels of this compound were determined as a function of time and dose in male Sprague-Dawley rats weighing approximately 200 g.

The assay procedure is based on the extraction of mescaline from biological tissues and fluids and on its reaction with dansyl chloride. The tissue (approximately 1–2 g) was homogenized in 3 volumes of 1 *N* HCl. To the homogenate or plasma (1.0 ml of plasma and 3 ml of 1 *N* HCl), 1.5 ml of 5 *N* NaOH and 30 ml of toluene were added. After shaking and centrifugation for 10 min, 25 ml of toluene were removed and shaken with 1.5 ml of 0.5 *M* boric acid for 10 min. After centrifugation for 10 min, 1 ml of the boric acid was combined with 1 ml of 0.1 *M* borax solution and 0.02 ml of dansyl chloride (10 mg/ml of acetone) and heated in a boiling water bath for 15 min. After cooling the samples were shaken with 1.5 ml of chloroform for 10 min, and the organic phase was read in an Aminco-Bowman Spectrofluorophotometer at 490 nm (activation 338 nm). Tissue samples from untreated animals with and without the addition of a known amount of mescaline served as internal standards or blanks, respectively.

The sensitivity of the procedure is approximately 0.5 µg/g or ml of sample and the recovery is between 65 and 80%. Fluorescence of extracts obtained from untreated animals was only slightly higher than that of 'water' blanks. Verification of the specificity of the method was obtained by thin layer chromatography[5] and by the BRODIE distribution[6].

After i.p. injection of mescaline (Table I), the compound appeared rapidly in plasma and liver and most of it disappeared within 2 h. Mescaline entered the brain slowly, showed peak levels at 30 min, and could still be detected after 210 min. The ratio of the concentrations in brain and plasma at peak levels was approximately 0.6. An increase in the dose injected (Table II) produced higher plasma and tissue levels with the exception of brain in which saturation was reached at a dose of 40 mg/kg. In separate experiments it was shown that brain levels of mescaline did not exceed 2.7 mg/g 60 min after injection of 80 mg/kg. These results might indicate that mescaline does not cross the blood-brain-barrier easily and is stored in the CNS at specific sites with a limited capacity.

Our data agree with those of DENBER and TELLER[7] who found cortical peak levels of approximately 1 µg/g 40 min after the injection of 10–18 mg/kg into female

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Table I. Concentrations of mescaline in rat brain liver, and plasma as a function of time

	Min after injection					
	5	15	30	60	120	210
	µg/g or ml					
Brain	n.d.	2.1 ± 0.4	3.2 ± 0.7	1.5 ± 0.9	1.25 ± 0.5	1.4 ± 0.8
Liver	35.5 ± 6.8	31.3 ± 5.9	22.1 ± 2.1	6.8 ± 2.2	3.0 ± 0.3	–
Plasma	3.5 ± 0.3	3.1 ± 0.8	4.9 ± 2.0	1.9 ± 1.0	n.d.	–

Each value is the mean ± the standard deviation from at least 3 animals. Rats received 40 mg/kg of mescaline × hemisulfate i.p. n.d., not detectable.