at 10, 20, 40, or 50 mg/kg (i.p.). *In no experiment* did phenitrone alter the ataxic state produced by THC or abolish its characteristic effects (Grunfeld & Edery, 1969).

The results of these three experiments, contrary to those of Kudrin & Davydova demonstrate that phenitrone is ineffective in the blockade of THC in dogs and mice. It seems improbable that these differences could be due to our use of THC by injection and their use of hashish extract by inhalation.

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Potentiation of responses to and inhibition of extraneuronal metabolism of catecholamines by veratrine

Uptake into neuronal structures is generally regarded as the dominant mechanism limiting the magnitude and duration of catecholamine action at the receptors of sympathetically innervated effectors (Trendelenburg, 1966; Iversen, 1971; Axelrod, 1971). Recent work has demonstrated, however, that the action of noradrenaline and adrenaline in vascular tissue is terminated by their penetration of effector cell membranes and subsequent metabolism (Kalsner, 1966; Kalsner & Nickerson, 1969a, b; Kalsner, 1971). In addition, the sensitization of responses to sympathomimetic amines by several diverse groups of agents (e.g. steroids, haloalkylamines, methylxanthines) has been linked to inhibition of amine inactivation in effector cells (Kalsner & Nickerson, 1969b; Kalsner, 1969a, b; Kalsner, 1971). We wish to report the finding that veratrine (cevadine), a veratrum alkaloid, also enhances responses of arterial strips to adrenaline and noradrenaline and inhibits the extraneuronal metabolism of these amines.

Rabbit aortic strips were prepared for isotonic recording as described previously (Kalsner & Nickerson, 1968a). The strips were suspended under 2 g tension at 37° in drain-out muscle chambers containing Krebs-Henseleit (Krebs) solution and contractions and relaxations were recorded on a slowly-moving kymograph drum (1·8 mm/min). The procedure of oil-immersion has been described in detail (Kalsner & Nickerson, 1968a). Aortic strips were contracted in the aqueous medium and after responses had plateaued the muscle chambers were emptied and filled with warmed (37°) and oxygenated (95% O_2 - 5% CO_2) mineral oil and relaxations recorded. The purpose of oil-immersion is to eliminate diffusion of agonist from the tissue into the aqueous bathing medium. Relaxation in oil is a measure of the rate of inactivation (termination of action) by intrinsic mechanisms (Kalsner & Nickerson 1968a, b). Concentrations of (−)-noradrenaline and adrenaline bitartrates, (±)-

methoxamine, (±)α-methylnoradrenaline and (−)-phenylephrine hydrochlorides and (−)-synephrine are referred to as the weight of the base and veratrine (cevadine) sulphate (Nutritional Biochemicals Co.) is referred to as the weight of the salt in g/ml in the muscle chambers. Monoamine oxidase (MAO) was inhibited by exposing strips to iproniazid (100 or 200 μg/ml) or pargyline (20 or 50 μg/ml) for 30 or 15 min, respectively, followed by a 30 or 15 min period with frequent washes before agonist administration. Catechol-*O*-methyltransferase (COMT) was inhibited with tropolone (10 μg/ml) or U-0521 (3′,4′-dihydroxy-2-methyl propiophenone) (10 μg/ml) or pyrogallol (3 μg/ml) administered in the presence of agonist as previously described (Kalsner, 1971). Reserpine (0·5 mg/kg) was administered intramuscularly about 18 h before death. Mean data are shown with their standard errors.

Strips were contracted by adrenaline (3 or 10 ng/ml) and after responses had reached plateau values veratrine was added to the chambers. Response magnitude was increased by 0·3 μg/ml of veratrine and maximal enhancement was usually evident at 3 μg/ml of the alkaloid. The potentiation was equivalent to increasing the bath

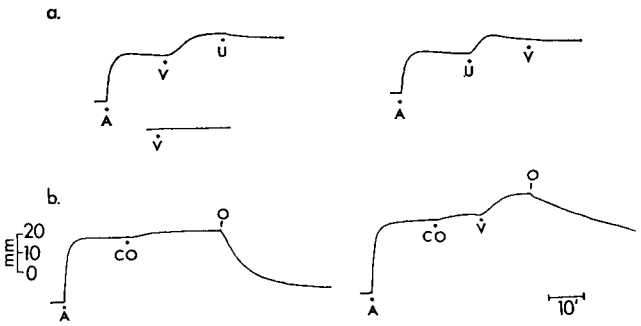

FIG. 1. Effects of veratrine on responses to and inactivation of adrenaline in aortic strips. (a), Strips from the same aorta contracted by adrenaline (A) (3 ng/ml) and exposed to veratrine (V) (3 μg/ml) and U-0521 (U) (10 μg/ml) in either sequence. Lower trace shows the lack of effect of (V) on uncontracted strip. (b), Strips from the same aorta pretreated with iproniazid (100 μg/ml) and contracted by adrenaline (A) (10 ng/ml). Left, strip exposed to cocaine (CO) (10 μg/ml) before oil immersion; right, strip exposed to (CO) followed by veratrine (3 μg/ml) and oil immersion.

Table 1. *Effects of veratrine on responses to sympathomimetic amines.*

Agonist (ng/ml)	No. of strips	Contraction (mm)	Increment (mm) due to veratrine
Adrenaline (3)	10	24·1 ± 1·6	10·1 ± 0·9
	*4	22·3 ± 1·2	10·0 ± 0·7
	†9	29·8 ± 0·8	10·6 ± 1·2
Adrenaline (10)	3	31·5 ± 1·3	9·5 ± 0·5
Noradrenaline (3)	7	27·2 ± 1·2	3·6 ± 0·8
α-Methylnoradrenaline (30)	5	17·3 ± 2·4	6·5 ± 1·0
Phenylephrine (10)	5	24·0 ± 2·1	0
(30)	2	28·0 ± 2·0	0
Methoxamine (100)	5	21·9 ± 4·3	0
Synephrine (500)	5	16·9 ± 2·7	5·0 ± 1·2
	*6	19·3 ± 1·1	0·8 ± 0·2
	†6	26·1 ± 1·2	3·6 ± 0·9
	*†6	24·8 ± 3·4	0·8 ± 0·3

* Strips pretreated with either pargyline or iproniazid to inhibit MAO as described in text.

† Rabbits pretreated with reserpine as described in text.

Strips were contracted by an agonist and after responses had plateaued exposed to veratrine (3 μg/ml).

concentration of adrenaline 2·3-fold. Pretreatment of rabbits with reserpine did not reduce the potentiating effect of veratrine (Table 1). Veratrine had no contractile effect of its own on aortic strips (Fig. 1a). Responses to noradrenaline were increased much less by veratrine and those to α-methylnoradrenaline to an intermediate extent (Table 1). Responses to the non-catecholamines methoxamine and phenylephrine were not increased by veratrine (3 μg/ml) but those to synephrine were significantly enhanced (Table 1). The potentiating effect of veratrine on catecholamine response amplitude was not reduced in the presence of cocaine (10 μg/ml), an inhibitor of neuronal uptake.

The order of potentiation of the catecholamines by veratrine (adrenaline>α-methylnoradrenaline>noradrenaline), as well as the magnitude of the enhancement, is similar to that obtained with inhibitors of COMT (Kalsner, 1969a, b; 1971). The possibility that veratrine decreased the rate of *O*-methylation of catecholamines in aortic strips was explored. Aortic strips were contracted by adrenaline (3 or 10 ng/ml) and about 10 min later exposed to veratrine (3 μg/ml) followed after an additional 10 min by a known inhibitor of COMT. The enhancing effects of tropolone, U-0521 and pyrogallol were completely abolished in the presence of veratrine in a total of 20 strips. Similarly, the effects of veratrine (3 μg/ml) on adrenaline response amplitude was usually completely blocked in aortic strips contracted by adrenaline and first exposed to a known COMT inhibitor. The mean residual increment produced by veratrine in the presence of tropolone, U-0521 or pyrogallol in 21 tests on 19 strips was 0·3 mm. Kymograph traces of typical experiments are presented in Fig. 1a. Interaction studies between COMT inhibitors and veratrine on α-methylnoradrenaline and noradrenaline-induced contractions yielded results similar to those described for adrenaline. In other experiments it was observed that pretreatment with iproniazid or pargyline to inhibit MAO, significantly reduced the enhancing effects of veratrine on responses to synephrine but not to adrenaline (Table 1).

More direct evidence of an effect of veratrine on the extraneuronal inactivation of sympathomimetic amines was provided using the technique of oil-immersion. It was previously reported that the major pathway terminating the action of adrenaline and noradrenaline in arterial tissue is *O*-methylation with MAO and uptake and storage

Table 2. *Relaxation in oil of aortic strips contracted by adrenaline (10 ng/ml).*

Treatment	No. of strips	Time to relax 50% (min)
Untreated	5	3·0 ± 0·5
U-0521 (10 μg/ml)	5	35·8 ± 1·8
Veratrine (3 μg/ml)	3	9·6 ± 0·5
Veratrine (10 μg/ml)	5	14·5 ± 0·4
U-0521 plus veratrine (3 μg/ml) ..	5	35·8 ± 2·9
†IPN plus cocaine (10 μg/ml) ..	7	3·8 ± 0·4
†IPN plus cocaine plus veratrine (3 μg/ml)	7	24·9 ± 1·3
IPN plus cocaine plus veratrine (10 μg/ml)	7	*21·3 ± 2·4

* Percent relaxation in 30 min.

† Pargyline was used in place of iproniazid (IPN) in some of these experiments.

Details of treatment conditions are given in text.

processes having secondary roles (Kalsner & Nickerson, 1969a; Kalsner, 1971). Aortic strips contracted by adrenaline (10 ng/ml) and after about 20 min exposed to oil, relaxed 50% in 3·0 min (Table 2). Strips contracted by adrenaline and exposed to the COMT inhibitor, U-0521, about 10 min before oil immersion, relaxed significantly slower. The increase in time to relax 50% compared to controls indicated that *O*-methylation accounted for 92% of the inactivation of this concentration of adrenaline. Combined inhibition of uptake and storage processes and MAO with cocaine and iproniazid produced a slight but not statistically significant slowing of relaxation in agreement with previous findings. Veratrine (3 μg/ml) added to the chambers containing strips contracted by adrenaline, about 10 min before oil immersion, significantly slowed relaxation; the shift of 3·2 times that of controls at 50% relaxation indicated a reduction of 69% in the tissues' capacity to inactivate adrenaline a higher concentration of veratrine gave an even greater effect (Table 2). Further evidence that veratrine decreased the rate of *O*-methylation of adrenaline were the findings that the alkaloid did not further slow relaxation in the presence of U-0521 and it had a significantly increased effect after inhibition of uptake and storage processes and MAO, as would be anticipated after elimination of alternate mechanisms of inactivation (Table 2). Typical kymograph traces are shown in Fig. 1b.

It is concluded that veratrine enhances responses to catecholamines by inhibiting their *O*-methylation. This coupled with the observation that the potentiation of responses to the MAO substrate, synephrine, by veratrine is markedly attenuated after inhibition of MAO suggests that the alkaloid rather than inhibiting a specific enzyme may produce a more generalized effect on sympathomimetic amine metabolism by impairing access of amine to extraneuronal sites of inactivation. This mechanism has been invoked previously to explain augmentation of response magnitude by apparently unrelated groups of agents (Kalsner & Nickerson, 1969b; Kalsner, 1969b, 1971). The variety of compounds which can enhance responses of vascular tissue to sympathomimetic amines by inhibition of their extraneuronal metabolism suggests an effective way of modulating the response level to catecholamines not only in blood vessels but in other tissues as well.

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