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Effects of acetone on responses of a number of tissues to biogenic amines*

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IN A RECENT report, Dikstein and Sulman[1] presented evidence that 25% acetone applied for 2 min could abolish contractions of rabbit uterus by acetylcholine (ACh) and norepinephrine and contraction of frog rectus abdominis muscle by acetylcholine. It was of considerable interest that phosphatidyl serine and lecithin could at times completely restore these responses, which otherwise were irreversibly abolished. The interpretation of these findings was that acetone had selectively extracted phospholipids from the ACh and norepinephrine receptors, thereby causing their inactivation, and that these phospholipids could be replaced from an exogenous source.

We have repeated some of these experiments on a number of isolated tissues: frog rectus abdominis, rabbit aortic strip, rabbit atria, guinea pig ileum and rat stomach fundus strip. Because of the possibility that acetone might exert effects on tissue elements other than the specific receptors, we tested each of the tissues treated with acetone (except atria) with KCl, a muscle stimulant which does not depend on any receptor mechanism.

Each of the tissues were bathed for 2 min in 25% acetone (Spectroquality, Fisher) diluted in the appropriate physiological solution. The tissues were washed exhaustively and tested with a number of agonists: serotonin, histamine and norepinephrine for the aortic strip; serotonin, ACh, angiotensin and bradykinin for the stomach; ACh, bradykinin, and angiotensin for the ileum; ACh and norepinephrine for the atria. Phosphatidyl serine (Mann Research Laboratories, synthetic) was used as described by Dikstein and Sulman. After acetone treatment, the phospholipid was applied at a final concentration of 10 μg/ml for 2 or 10 min, followed by wash and retesting of the pertinent agonists. Control responses were determined before application of the acetone. Ten atria and six or more preparations of each of the other tissues were studied in the above manner.

The results may be summarized as follows: Acetone, under the described conditions, completely abolished responses of the aortic strip and ileum to all agonists including KCl. These agonists at 10-fold higher concentrations than those which gave a maximum contraction in the controls were ineffective in eliciting any response. Phosphatidyl serine applied for 2 or 10 min failed to restore contractility to any agonist.

Acetone induced spontaneous contractions in the stomach strips, making testing of the various agonists somewhat difficult. Nevertheless, it was apparent (Table 1) that only KCl, at 10 times higher concentration than the control, was able to produce a contraction; this averaged about 60 per cent of the control maximum. Responses to all the other agonists were completely abolished and were not restored by application of phosphatidyl serine for 2 or 10 min.

Rabbit atria stopped beating within a few seconds after application of the acetone. The beat slowly recovered to a slight extent upon wash; recovery was not improved by norepinephrine or phosphatidyl serine. However, application of calcium (1·3 mg/ml) oro uabain (0·8 μg/ml) for a few minutes resulted eventually in an almost complete restoration of the spontaneous beat. At this time, responses to control concentrations of norepinephrine and ACh could readily be elicited. These averaged 55 per cent and 75 per cent of control responses to norepinephrine and ACh respectively.

On frog rectus, we were unable to confirm the results reported by Dikestein and Sulman; abolition of the ACh response by acetone was not restored by phosphatidyl serine. Moreover, acetone completely and irreversibly abolished the response to very high concentrations of KCl, indicating the probable destruction of the conducting membrane, contractile elements of the tissue, or both.

The following conclusions may be drawn from this study.

1. When employing an agent such as acetone or others (e.g. heat, e.g. enzymes, etc.) for the purpose of modifying muscle receptors, it is essential to examine responsiveness of the tissue to a stimulant such as KCl or $BaCl_2$ in order to be certain that changes in responses are due to selective action upon the specific receptors and not to generalized tissue depression. Without such a control,

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it might have been concluded that acetone had destroyed the various receptors present in guinea pig ileum, aortic strip and frog rectus abdominis. As it turned out, use of acetone revealed nothing about the receptors of these tissues.

TABLE 1. RESPONSES OF RAT FUNDUS STRIPS (n = 6) TO VARIOUS AGONISTS AFTER TREATMENT WITH ACETONE AND PHOPHATIDYL SERINE (PS)

	Before acetone		After acetone			
				Contraction (mm)*		
	Concn of agonist	Contraction (mm)*	Concn of agonist	After washing	PS 2 min	PS 10 min
KCl	0·75 mg/ml	119 ± 10	7·5 mg/ml	75 ± 23	72 ± 37	78 ± 23
ACh	1·25 ng/ml	96 ± 20	12·5 ng/ml	3 ± 3	6 ± 8	3 ± 5
Serotonin	1·25 ng/ml	112 ± 15	12·5 ng/ml	7 ± 7	10 ± 13	3 ± 5
Bradykinin	2·5 ng/ml	78 ± 35	25 ng/ml	4 ± 7	3 ± 6	10 ± 12
Angiotensin	2·5 ng/ml	100 ± 19	25 ng/ml	5 ± 7	4 ± 7	9 ± 14

* Each value is given as mean ± S.E.

2. Tissues can have very different stabilities to agents employed to alter receptors; this was shown to be the case for acetone as well as for heat (Fleisch and Ehrenpreis, unpublished). It is evident, therefore, that a procedure designed to alter receptors must be evaluated on a number of tissues in order to fully assess its utility in this regard.

3. It is fairly likely that acetone, even when it did not depress a tissue nonspecifically (e.g. rabbit atria), fails to extract lipids or phospholipids from receptors. This was indicated by the rapidity of cessation of activity of the atria as well as by the fact that responses to ACh and norepinephrine could be readily elicited once the beat was restored by Ca or ouabain. The latter finding suggests rather that acetone selectively inactivates the excitation–contraction coupling mechanism of atria. Further studies along these lines could provide insight concerning the macromolecular components involved in such coupling.

4. Even when acetone seemed to inactivate receptors, as in the case of the rat stomach, abolition of responses to the various biogenic amines is not considered to be due to alteration of their receptors by a mechanism involving lipid or phospholipid extraction but rather to some other effect. We have obtained extensive evidence that in this tissue the receptors for each of the agonists examined can be altered by heat or urea, suggesting that they are protein in nature.[2–5] Acetone is known to be a potent protein denaturant. Thus the acetone inactivation of the receptors of this tissue is entirely consistent with our earlier findings involving the use of two entirely different receptor-modifying procedures. However, it is evident that when used in the manner described, acetone is far less selective than either heat or urea insofar as receptor modification is concerned. It is conceivable that under other conditions, e.g. lower concentrations or reduced temperature, differential denaturation of receptors by acetone could be achieved.

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