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Comparative effects of sodium azide and aminophylline on the rat isolated uterus during muscle activation

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Sodium azide is a strong inhibitor of the tonic component of contraction produced by oxytocin, whereas aminophylline produces almost equal inhibition of all types of activation of the isolated rat uterus. Both substances inhibited the spontaneous rhythmic activity of the uterus. The effect of sodium azide is easily reversed by calcium. The results are taken to indicate a complex relation between calcium and substances which stimulate metabolism either of cGMP (sodium azide) or cAMP (aminophylline) in producing relaxation of the isolated rat uterus.

Sodium azide regularly produced a concentration-dependent depression of the rat isolated uterus activated by oxytocin, by electrical stimulation and by a high concentration of KCl. This azide caused a strong inhibition of the tonic component of contraction produced by oxytocin. On the contrary, aminophylline produced almost equal inhibition of all types of activation of the isolated uterus. The spontaneous rhythmic activity was also inhibited by sodium azide and aminophylline. The effect of sodium azide was easily reversed by an increased concentration of calcium in the medium. It is concluded that substances which produce either an increase in the concentration of cyclic (c) GMP (sodium azide) or of cyclic (c) AMP (aminophylline) are capable of producing relaxation of the rat isolated uterus and of depressing spontaneous rhythmic activity. The results are compatible with the view that a complex relation between cyclic nucleotides and calcium is implicated in the smooth muscle relaxation.

Materials and methods

All the experiments were on rat isolated uteri, taken from Wistar rats pretreated with stilboestrol (1·5 mg kg^{-1} intramuscularly, 24 h before the experiment). The preparation was suspended in an organ bath of 15 ml capacity. The uterus was immersed in de Jalon's solution of the following composition (in mM): NaCl 153; KCl 5·63; $CaCl_2$ 0·54; $NaHCO_3$ 5·9; glucose 2·77. This solution was bubbled with a mixture of 97% O_2 and 3% CO_2. Both the isometric contractions and the spontaneous rhythmic activity were recorded using an Ugo Basile isometric transducer and displayed on a recording microdynamometer 7050 (Ugo Basile). The activation of the preparation was produced by electrical field stimulation (25 Hz, 2 ms, in 'packets' of 7 s duration), by high concentrations of KCl (50 mM) and by oxytocin (3·3 and 33 μU ml^{-1}). Preload of the preparation was about 1 g.

* Correspondence.

Results and discussion

Sodium azide depressed the activation of the preparation produced by KCl, electrical stimulation and oxytocin. In concentrations from 0·2–1·8 mM, sodium azide produced a significant depression and even complete block of the tonic component of contraction produced by oxytocin and of the spontaneous rhythmic activity. Significantly higher concentrations of azide (up to 16·2 mM) produced only a small depression of the phasic component of contraction caused by oxytocin (Fig. 1). The effect on the phasic contractions produced by electrical stimulation was between these two extremes.

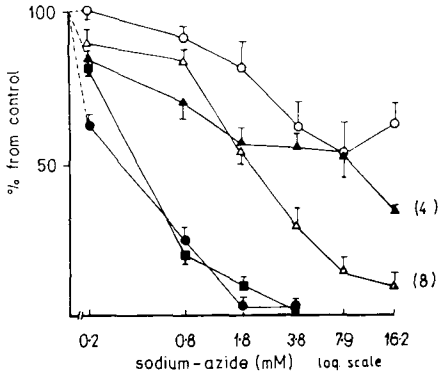

FIG. 1. The effect of increasing concentrations of sodium azide on the percentage reduction of phasic component of contraction produced by oxytocin (○) and electrical stimulation (△), on the tonic components of contractions produced by oxytocin (●) and high concentration of KCl (▲), and also on the spontaneous rhythmic activity of the rat isolated uterus (■). Every point is the mean of 5–8 experiments ± s.e.m.

Aminophylline in micromolar concentrations produced an almost equal inhibition of all types of activation of the preparation, e.g. tonic components of contractions caused by oxytocin and KCl, phasic components caused by oxytocin and electrical stimulation and spontaneous rhythmic activity (Fig. 2). Adenosine (2–100 μM) did not antagonize the relaxing effect of aminophylline.

Both aminophylline and sodium azide depressed the spontaneous rhythmic contractions and, if used in sufficiently high concentrations, produced a complete cessation of the spontaneous rhythmic activity. This

effect was easily reversed by doubling the concentration of calcium in the bath.

Various nitro compounds have been found to increase cGMP levels in smooth muscle (Böhme et al 1978). This effect is attributable to an increase in the guanylate cyclase activity (Murad et al 1978). Schultz et al (1977) have suggested that cGMP may act as a mediator of relaxations in smooth muscles. The relaxant effect of nitroglycerol was significantly related to an increase in the cGMP content of the artery (Axelsson et al 1979). On the other hand, Janis & Diamond (1981) found that, in the guinea-pig taenia coli large contractions can be obtained with only small increases in cGMP, and under other conditions, large increases in cGMP can be obtained with little increase in isometric tension. Some nitrates have been described as calcium-channel-blocking agents (Triggle & Swamy 1980). Thus, the mechanism of the relaxing action of sodium azide (and probably other nitro compounds) does not seem to be resolved.

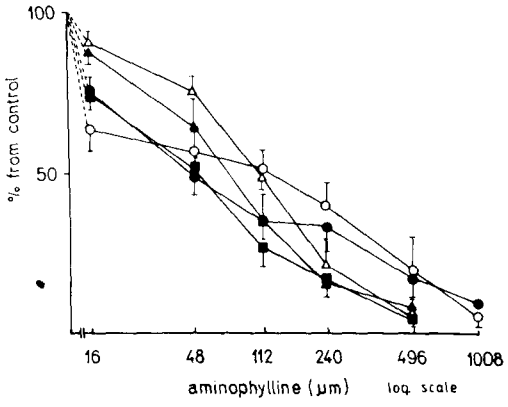

FIG. 2. The effect of increasing concentrations of aminophylline on the percentage reductions of contraction of the rat isolated uterus produced by oxytocin (○) = phasic component of contraction, oxytocin tonic (●) = tonic component of contraction, by electrical stimulation (△) (phasic component), by 50 mM KCl (▲) (tonic component of contraction) and also on the spontaneous rhythmic activity (■). Every point is the mean of 5–7 experiments ± s.e.m.

Various types of activation of the rat isolated uterus were also easily antagonized by aminophylline, which is known to increase the cAMP content of smooth muscle. Adenosine receptors most probably are not involved in the action of aminophylline, because adenosine itself did not antagonize the relaxing action of aminophylline during various types of smooth muscle activation. The increase in cAMP may exert an effect in decreasing the level of free calcium in the myoplasm. This may be achieved by increasing Ca-binding in the intracellular stores and plasma membrane, or by reducing the influx of Ca through the plasma membrane, or by stimulating the active extrusion of Ca (Bülbring 1981; Walsh et al 1979).

We found that sodium azide produced a stronger inhibition of the tonic component of contraction caused by oxytocin than of the phasic component of contraction caused by oxytocin. The same type of response was observed after calcium-channel-blocking agents (Varagić et al 1984). This indicates that some change in the metabolism of calcium might be implicated in the relaxing actions of sodium azide. If this is so, then the phasic and tonic components of contraction caused by oxytocin are realized through different types of calcium channels and these are differently affected by sodium azide. Evidently, the channel through which the tonic component of contraction is realized is much more sensitive towards the action of sodium azide than the channel through which the phasic component is carried out.

Our experiments show that both sodium azide and aminophylline produce a depression of the spontaneous rhythmic phasic contractions. This inhibitory effect was found to be easily reversed by an increase in extracellular calcium. Previously it was shown that the calcium channel blockers verapamil and nifedipine produced a block of the spontaneous rhythmic activity even if used in low concentrations (Varagić et al 1984). Our experiments therefore indicate that an inactivation of calcium might be a primary factor in stopping the spontaneous rhythmic activity. However, the present experiments do not exclude possible relations between cyclic nucleotides and calcium in producing changes in the spontaneous rhythmic activity of the isolated rat uterus.

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REFERENCES

Axelsson, K. L., Wirkberg, J. E. S., Andersson, R. G. G. (1979) Life Sci. 24: 1779–1786

Böhme, E., Graf, H., Schultz, G. (1978) Adv. Cycl. Nucl. Res. 9: 131–143

Bülbring, E. (1981) in: Bülbring, E., Brading, A. F., Jones, A. W., Tomita, T. (eds) Smooth muscle—an assessment of current knowledge. Edward Arnold, London, pp. X–XII

Janis, R. A., Diamond, J. (1981) Eur. J. Pharmacol. 70: 149–156

Murad, F., Mittal, C. K., Arnold, W. P., Katsuiki, S., Kimura, H. (1978) Adv. Cycl. Nucl. Res. 9: 145–158

Schultz, K. D., Schultz, K., Schultz, G. (1977) Nature (Lond.) 265: 750

Triggle, D. J., Swamy, V. C. (1980) Chest, Suppl. 78: 174–178

Varagić, V. M., Milovanović, S. R., Srkalović, G. (1984) Arch. Int. Pharmacodyn. 270: 79–87

Walsh, D. A., Clippinger, M. S., Silvaramakrishnan, S., McCullough, T. M. (1979) Biochemistry, 18: 87–95