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ACTIONS OF SODIUM ASCORBATE ON SMOOTH MUSCLE

BY

W. DAWSON,* B. A. HEMSWORTH † AND M. A. STOCKHAM ‡

From the Department of Pharmacology, School of Pharmacy, University of London

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Ascorbic acid is frequently added to food and drug preparations. There have, however, been very few studies on the pharmacological activity of the vitamin, although its anti-scorbutic properties have been studied in great detail.

Large doses of ascorbic acid inhibit the bronchospasm induced in guinea-pigs by spasmogens such as histamine, and exert a beneficial effect in pathological states such as anaphylaxis (Dawson & West, 1965a). Contractions of intestinal smooth muscle are also inhibited by large concentrations of the vitamin (Dawson, Hemsworth & Stockham, 1965). Further studies of the pharmacological effects of ascorbic acid on smooth muscle were therefore considered of interest.

METHODS

Bronchoconstriction in vivo

Guinea-pigs (300–500 g) were anaesthetized with chloralose (110 mg/kg intraperitoneally) and artificially ventilated by a constant volume pump through a tracheal cannula. Pressure in the trachea was recorded using a transducer system. Drugs were injected into a jugular vein.

Isolated guinea-pig ileum preparation

A segment of terminal guinea-pig ileum (2–2.5 cm) was suspended in aerated Tyrode solution at 32° C in a 10 ml. bath and contractions were recorded isotonically using a frontal writing lever with a magnification of 4 and a load of 1 g. Drugs were dissolved in 0.9% saline. Ascorbic acid was dissolved in distilled water and neutralized to pH 6–7 with sodium bicarbonate, unless otherwise stated in the text. The dose cycle for acetylcholine, 5-hydroxytryptamine and histamine was 3 min and the contact time 30 sec. The sodium ascorbate was mostly left in contact with the tissue for 5 min and then washed out before the next dose of spasmogen. Doses of all drugs are expressed in g/ml. bath volume.

Transmural stimulation of guinea-pig ileum

The method described by Paton (1955) was used.

* Present address: Department of Pharmacology, British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey.

† Present address: University Department of Pharmacology, Cambridge.

‡ Present address: Sherrington School of Physiology, St. Thomas's Hospital Medical School, London S.E.1.

Diet deficient in ascorbic acid

Guinea-pigs were made deficient in ascorbic acid by feeding a diet of autoclaved Rank SG1 pellets, supplemented with vitamins A, B complex and D. Other animals received the same diet with ascorbic acid (50 mg/guinea-pig/day) included in the drinking water.

RESULTS

Effect of sodium ascorbate on bronchospasm in guinea-pigs

Intravenous histamine (5 μg/kg) produced a marked bronchoconstriction in guinea-pigs as indicated by an increase in tracheal pressure with constant volume lung inflations (Fig. 1). This bronchoconstriction was inhibited by sodium ascorbate (200 mg/kg) given 1 min previously. After 15 min, the response to histamine was potentiated and then, after a further 15 min, it had returned to control levels. Bradykinin (10 μg/kg), 5-hydroxytryptamine (5 μg/kg) and acetylcholine (5 μg/kg) also induced bronchoconstriction which was first inhibited and then potentiated by ascorbate. The potentiation and inhibition of bronchoconstriction induced by these spasmogens were not modified by depleting the tissues of catecholamines and 5-hydroxytryptamine with reserpine (5 mg/kg daily for 2 days), nor did β-blocking drugs, or acute adrenalectomy, have any effect (Dawson & West, 1965b).

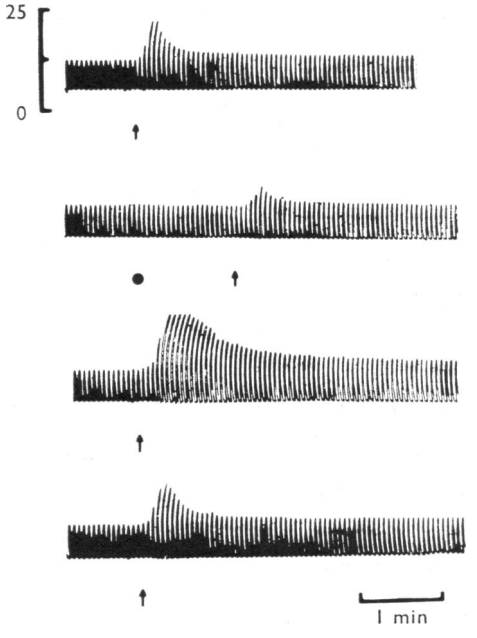

Fig. 1. This is a continuous record showing tracheal pressure in mm Hg in guinea-pigs. At the arrows 5 μg/kg histamine and at the dot sodium ascorbate (200 mg/kg) were injected intravenously. An increase in size of response represents bronchoconstriction. Note the initial inhibition of the histamine response followed by a marked potentiation.

Effect of sodium ascorbate on isolated guinea-pig smooth muscle

Contractions produced by acetylcholine (4×10^{-9}) increased three-fold after the addition of a single dose of sodium ascorbate (5×10^{-4}) (Fig. 2a). Increasing the dose of ascorbate to 10^{-3} resulted in an initial block which was followed by a two-fold increase in response to acetylcholine (Fig. 2b). Increasing the dose further to 5×10^{-3} produced a contraction *per se* but partially inhibited the effect of spasmogen for 15 min before a three-fold potentiation occurred (Fig. 2c). A single dose of 10^{-2} first produced a small contraction and then blocked the acetylcholine response, which failed to recover to the control level (Fig. 2d). Similar results were obtained using histamine and 5-hydroxytryptamine as spasmogens.

The degree of blockade of the effect of the spasmogens by ascorbate was a function of concentration and contact time of the vitamin—for example, 10^{-2} ascorbate for 5 min

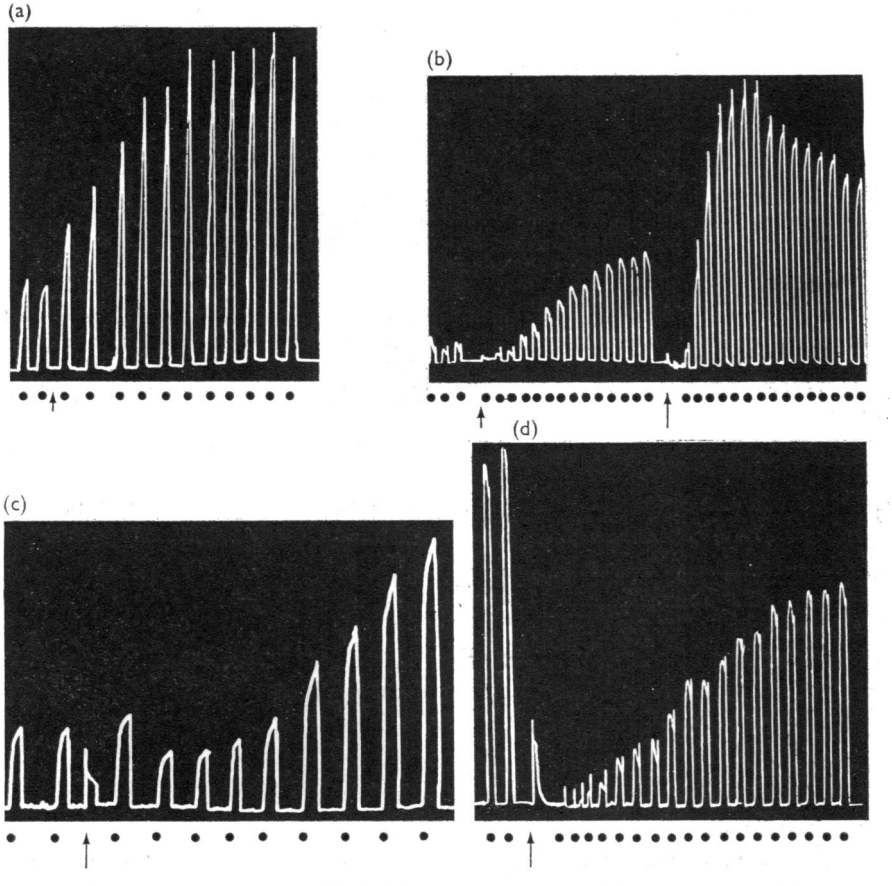

Fig. 2. Contractions of the guinea-pig ileum: at the dots acetylcholine 4×10^{-9}. (a) At the arrow 5×10^{-4} ascorbate. (b) At the arrows 10^{-3} ascorbate. (c) At the arrows 5×10^{-3} ascorbate. (d) At the arrow 10^{-2} ascorbate. Note the potentiation with the smaller doses and the inhibition with the larger doses of ascorbate.

gave a similar degree of inhibition to 10^{-3} ascorbate for 30 min. These values may be compared with 10^{-3} ascorbate for 5 min (Figs. 2b and d).

Sodium ascorbate produced contraction of guinea-pig ileum giving a straight line log dose/response curve, and tachyphylaxis was observed at a dose level of 1.6×10^{-2}, with subsequent recovery after a 15-min rest period (Fig. 3).

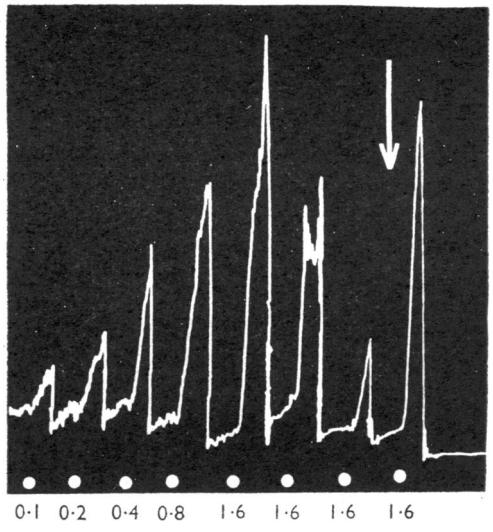

Fig. 3. Contractions of guinea-pig ileum with sodium ascorbate using a 3-min dose cycle: at the dots sodium ascorbate (doses $\times 10^{-2}$ g/ml.) was added to the bath and left in contact for 30 sec and then washed out. Note the tachyphylaxis to the concentration 1.6×10^{-2}. At the arrow the ascorbate was washed out and after a 15-min wait 1.6×10^{-2} ascorbate was again added to the bath.

This effect was probably not due to the antioxidant action of the vitamin, as sodium metabisulphite and thiourea had no spasmogenic effect themselves, nor did they affect the response of the tissue to spasmogens in low doses, although contractile responses were irreversibly blocked by large doses.

Ascorbate also produced potentiation (Fig. 4a) or blockade (Fig. 4b) or both blockade and potentiation (Fig. 4c) of the response to supramaximal stimulation of the transmurally stimulated guinea-pig ileum.

Effect of blocking drugs on the direct spasmogenic effect of sodium ascorbate on guinea-pig ileum

The spasmogenic action of sodium ascorbate was not modified by atropine (10^{-8}), mepyramine (5×10^{-7}) and 2-bromolysergic acid (10^{-7}) which respectively antagonized the responses to acetylcholine, histamine and 5-hydroxytryptamine. However, the sodium ascorbate response was blocked by papaverine (10^{-3}), a non-specific spasmolytic drug.

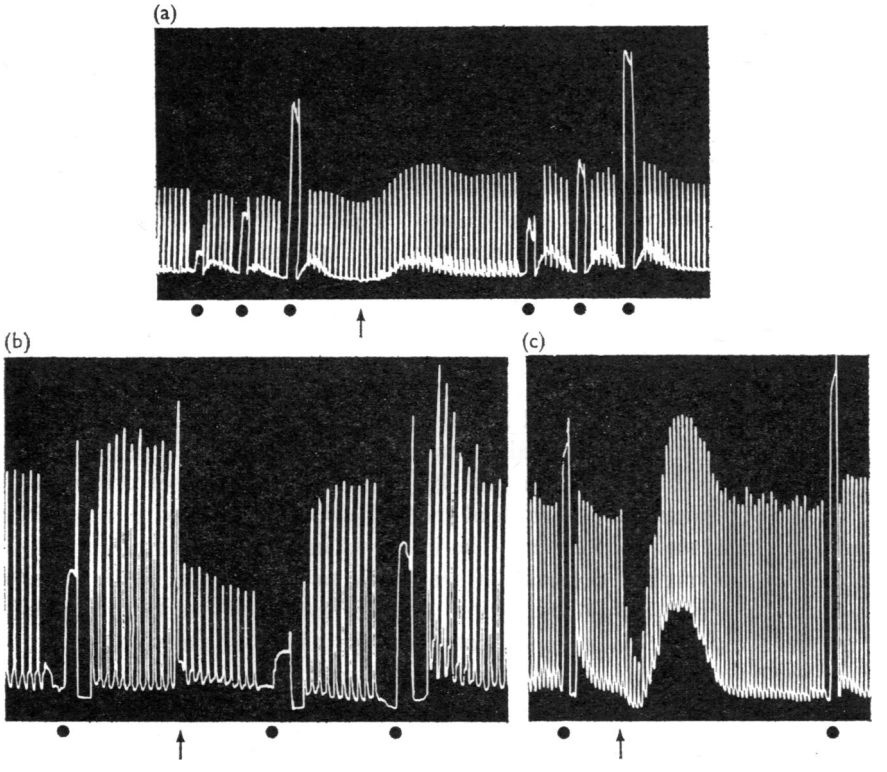

Fig. 4. Contractions of guinea-pig ileum elicited by transmural stimulation. (a) At the dots 10^{-8}, 2×10^{-8}, 4×10^{-8}, acetylcholine respectively and 10^{-3} ascorbate at the arrow. (b) At the dots 8×10^{-9} acetylcholine and 10^{-2} ascorbate at the arrow. (c) At the dots 8×10^{-3} acetylcholine and 5×10^{-3} ascorbate at the arrow. Note the potentiation with the smaller doses and the inhibition with the larger doses of ascorbate.

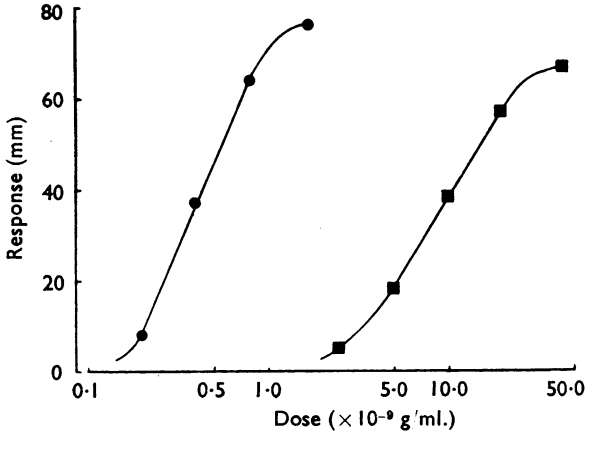

Fig. 5. Log dose/response curves for acetylcholine using terminal guinea-pig ileum preparations from guinea-pigs fed the complete diet (●——●) and from guinea-pigs maintained 21 days on the ascorbic acid deficient diet (■——■).

Effect of deficiency of ascorbic acid on the sensitivity of guinea-pig ileum to spasmogens

The log dose/response curves obtained with acetylcholine on segments of ileum from 21-day ascorbic acid deficient and control guinea-pigs fed the complete diet are shown in Fig. 5. This ten-fold difference was also seen when comparisons of other segments of ileum were made—for example, the duodenal region or the midileal region—of deficient and control animals. The sensitivity of the deficient tissue could be restored either by adding sodium ascorbate to the isolated tissue (5×10^{-4}) or by adding ascorbic acid to the diet (50 mg/guinea-pig/day). Similar results were obtained with other spasmogens.

DISCUSSION

Although the doses of ascorbate used in these experiments are large, children and adults ingesting certain foods and vitamin preparations may have an intake approaching 200 mg/kg, which was the dose used in the guinea-pig bronchospasm experiment. It is also important to note that to maintain sensitivity of the ileum to spasmogens, ascorbic acid was needed in the diet, and in fact, by ensuring that the diet contained enough ascorbic acid for maintenance of health of the guinea-pig, sensitivity of intestinal muscle to spasmogenic drugs was maintained. The intestinal muscle from ascorbic acid-deficient guinea-pigs shows a marked decrease in sensitivity to spasmogens, well before clinical signs of scurvy are induced. In addition, sodium ascorbate exerts several effects on smooth muscle which are dose dependent: it may inhibit or potentiate the action of spasmogens, or have a direct stimulant action *per se*.

Ascorbic acid has been used clinically for many years in the treatment of various conditions, including many concerned with muscular disorders. Hirata & Suzuki (1935, 1937) described a deficiency of ascorbic acid in the cerebrospinal fluid of patients suffering from progressive muscular atrophy; the administration of ascorbic acid improved this condition, increasing the glycogen content of muscle. Piéry, Cordier & Enselme (1940) showed that deposition of liver glycogen was also augmented. Spitzer (1947) found that small doses of ascorbic acid were useful in the first stage of labour as an oxytocic agent, and often potentiated a small dose of pitocin, whereas larger doses of ascorbic acid had little effect. Our findings—that larger doses of ascorbate are inhibitory—may explain this observation.

Many workers have used ascorbic acid as an antihistamine in the treatment of allergic reactions, with varying degrees of success. Rosa & Parenti (1950) used the vitamin in bronchial asthma and urticaria with some success, but Naranjo (1952) and Herxheimer (1955) obtained no protection against anaphylaxis in experimental animals. Hochwald (1935), van Niekerk (1937), Aron (1948) and Dawson & West (1965a) all showed a short-term protective action of ascorbic acid against anaphylaxis in guinea-pigs. These protective effects have usually been obtained in guinea-pig and man in allergic conditions where contraction of the bronchial smooth muscle is of prime importance and it is suggested that the protection is due to a direct action of the vitamin on the smooth muscle.

Ascorbic acid may have a synergistic effect when administered with antihistamines against allergic phenomena (Dawson & West, 1965a) since rat anaphylaxis is completely inhibited by pretreatment with a mixture of these drugs. This result may be correlated with the blocking action of ascorbate on guinea-pig smooth muscle preparations against the agonist histamine as well as against other agonists such as acetylcholine and

5-hydroxytryptamine. Preliminary clinical studies show that ascorbic acid itself can partially relieve the spasm of bronchial smooth muscle in both hay-fever and bronchial asthma.

SUMMARY

1. Sodium ascorbate has a direct stimulant action on smooth muscle.

2. Spasmogenic responses are potentiated by sodium ascorbate in low concentrations and are inhibited by high concentrations of this substance.

3. The actions of sodium ascorbate are not modified by atropine, mepyramine, 2-bromolysergic acid or reserpine.

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