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Cooling-induced augmentation of the contractile response of the golden hamster tracheal muscle to substance P in-vitro

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The effect of temperature on the substance P-induced contraction of the isolated tracheal strip-chain preparation from the golden hamster has been examined. A decrease of bath temperature from 37 to 20 °C augmented the contractile response of the trachea caused by substance P. Similar cooling-induced augmentation was observed in the contractile responses to caffeine and carbachol, but not to potassium chloride. The increased responsiveness with lowered temperature of the trachea to substance P may be due to the acceleration of Ca^{2+} release from an intracellular storage site.

It is well known that exercise, especially in cold air, increases airway resistance in patients with asthma. This exercise-induced bronchoconstriction has been thought to result from an increase in muscle sensitivity to acetylcholine due to the temperature reduction in the airway smooth muscle layer (Black et al 1984; Ishii & Shimo 1985). This hypothesis is based on the findings that (i), during exercise, the temperature in the oesophagus adjacent to the trachea is decreased (Deal et al 1979) and (ii), in the isolated airway smooth muscles of man (Murlas et al 1982; Black et al 1984) or animals (Pandya 1980; Ishii & Shimo 1985), lowering of the bath temperature from 37 to 20 °C augments the contractile response to cholinergic nerve stimulation or to acetylcholine. However, the effect of muscarinic antagonists on exercise-induced bronchoconstriction is less certain. Some workers have found that exercise-induced bronchoconstriction can be inhibited by treatment with muscarinic antagonists (Simonsson et al 1972; Tinkelman et al 1976; Chen et al 1981), whereas others have suggested that these drugs are ineffective (Chan-Yeung et al 1971; Deal et al 1978).

Recently, it has been demonstrated that nerve fibres containing substance P are abundant in the smooth muscle layer of human airway (Lundberg et al 1984) and it is this that causes the contractile response in the isolated airway smooth muscles of man (Lundberg et al 1983) and animals (Nilsson et al 1977; Tanaka & Grunstein 1984). These findings raised the possibility that the increase in muscle tone caused by substance P may also be augmented by heat loss during exercise. In the present study, therefore, we examined the effect of cooling on substance P-induced contraction using the isolated tracheal muscle from the golden hamster.

Materials and methods

Male golden hamsters, 80–130 g, were killed by a blow on the head. The trachea (10 mm) was dissected, and cut into several rings of equal width (1·5 mm). Three of the randomized rings were tied together with silk thread to form a chain. The remaining rings were used to form a pair of strip-chain preparations. Each preparation was immersed in a 5 ml organ bath filled with Krebs bicarbonate solution of the following composition (mM): NaCl 118·1, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·0, $NaHCO_3$ 25·0 and glucose 11·1 (pH 7·3–7·4). Ca^{2+}-free Krebs solution was prepared by removing $CaCl_2$ from the Krebs bicarbonate solution and by adding ethylene glycol bis (β-aminoethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA, 0·1 mM). Both solutions always contained 100 μM hexamethonium to prevent the nicotinic activation of cholinergic nerves and were bubbled with 5% carbon dioxide in oxygen.

Changes in muscle tension were recorded on an inkwriting recticorder (RJQ 3006, Nihon Kohden) using a force displacement transducer (SB-1T-H, Nihon Kohden). The initial resting tension imposed on the preparation was 2 mN. Experiments were started after a 1 h equilibration period. The data obtained were expressed as mean ± s.e. mean. A two-tailed Student's *t*-test was used for statistical evaluation of the data. *P* values $<0·05$ were considered to be significant.

Drugs used were: substance P (Protein Research Foundation), carbachol chloride, diphenhydramine hydrochloride (Sigma), atropine sulphate, hexamethonium chloride, caffeine (Wako Pure Chem.) and tetrodotoxin (Sankyo). To prepare stock solutions, all drugs were dissolved in physiological saline (0·9% w/v NaCl). Drug concentrations in the text are expressed as final molar concentration in the organ bath. When KCl was used as a stimulant, the stated concentration excludes the KCl provided by the formulation of Krebs bicarbonate solution.

Results

Influence of cooling on substance P-, carbachol- or KCl-induced response. At 37 °C, the isolated tracheal strip-chain preparation of the golden hamster usually showed neither tone nor spontaneous activity. Substance P (10 μM) produced an increase in tension which had a rapid onset and reached a peak in about 1 min. Cooling of the bath temperature from 37 to 20 °C lengthened the time to peak tension (2–3 min) and augmented the amplitude of the substance P-induced response by about 120% (n = 7) (Fig. 1). The

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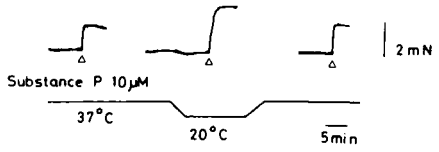

FIG. 1. Influence of cooling on the tension increase caused by substance P (10 μM) of the golden hamster isolated tracheal strip-chain preparation.

cumulative concentration-response curves for substance P (0·3–10 μM) at 37 and 20 °C are shown in Fig. 2. The contractile response to substance P was restored to the original level immediately on rewarming to 37 °C (Fig. 1). Similar cooling-induced augmentation was obtained from the experiment using carbachol (0·1–10 μM) (Fig. 2). However, cooling had little or no effect on the KCl (20–40 mM)-induced contractions.

In all experiments where the effect of cooling was examined, control experiments were carried out at the same time using paired tracheal strip-chain preparations. These experiments showed that the positions of the cumulative concentration-response curves for substance P, carbachol and KCl changed little when these curves were constructed every 30–50 min at 37 °C.

Effect of deprivation of extracellular Ca^{2+} and drugs on the responses to substance P, carbachol and KCl. At 37 °C, the contractile response of the tracheal strip chain caused by substance P (10 μM) was not affected by tetrodotoxin (0·5 μM, n = 4), atropine (1 μM, n = 4) or diphenhydramine (3 μM, n = 4). The results indicate that the substance P-induced contraction is mediated by a direct action on the smooth muscle. On the other hand, incubation of tissue with verapamil (1 μM) or with Ca^{2+}-free EGTA (0·1 mM) containing Krebs solution for 15 min caused some depression of the cumulative concentration-response curves for substance P (0·3–10 μM) and carbachol (0·1–10 μM), and almost abolished the KCl (20–60 mM)-induced responses (Fig. 3).

Effect of caffeine on basal tone. At 37 °C, caffeine (0·5–1 mM) had little or no effect on the basal tone of the tracheal strip chain. However, at 20 °C, it caused an increase in tension which was about 30% (n = 4) of the maximal response to carbachol.

Discussion

In golden hamster isolated tracheal muscle, lowering of the bath temperature from 37 to 20 °C augmented the contractile response to substance P. Similar cooling-induced augmentation was observed in the contractile response to carbachol but not to KCl.

We have previously demonstrated that the contractile response of rat isolated tracheal muscle to carbachol is augmented by cooling, and suggested that this cooling-induced augmentation is due to the acceleration of Ca^{2+} release from intracellular storage sites (Ishii & Shimo 1985). In the present experiment, both contractile responses of the golden hamster trachea to substance P and carbachol were relatively resistant to removal of extracellular Ca^{2+} or the Ca^{2+} entry blocker, vera-

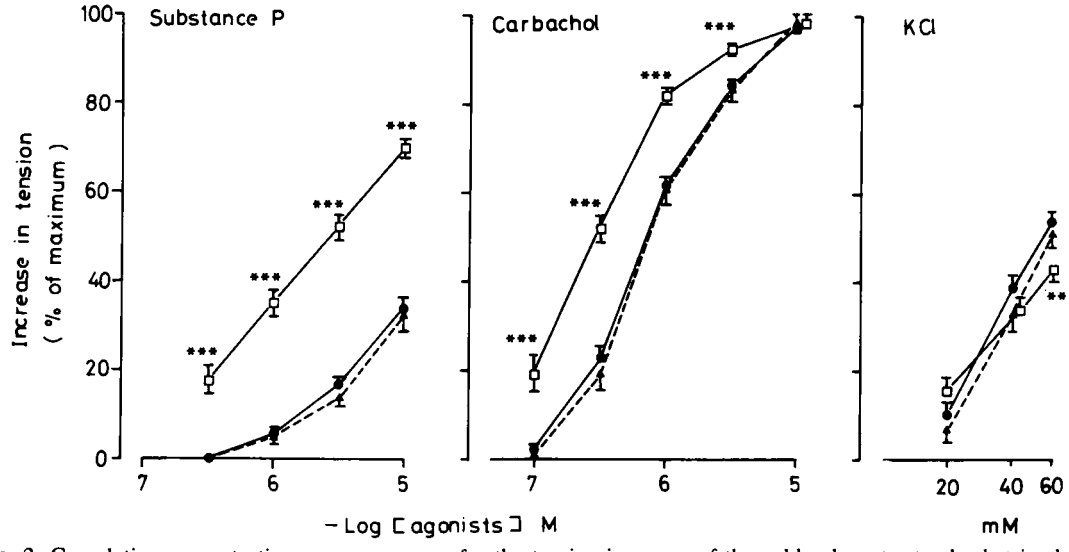

FIG. 2. Cumulative concentration-response curves for the tension increases of the golden hamster tracheal strip-chain preparations caused by substance P (0·3–10 μM), carbachol (0·01–10 μM) or KCl (20–60 mM) at 37 °C (●, ▲) and 20 °C (□). Concentrations of substance P greater than 30 μM were not tested. The ordinate scale represents the response as a percentage of the maximum response caused by carbachol at 37 °C (100%: 7·3 ± 0·4 mN). The symbols represent the initial concentration-response curve obtained at 37 °C (●) and the second concentration-response curve obtained at 37 °C (▲, time-matched control) or 20 °C (□). Rest intervals between curves were 20–30 min. Each point is the mean value from at least 7 experiments and the vertical bars represent s.e.m. $^{**}P < 0·01$; $^{***}P < 0·001$ when compared with the values of initial concentration-response curve (unpaired *t*-test).

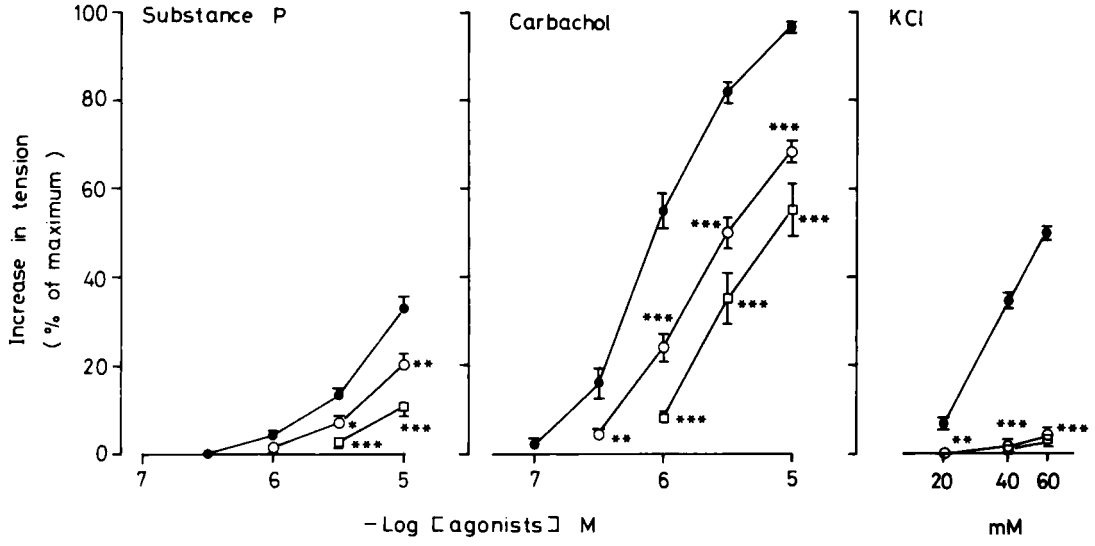

FIG. 3. The effects of Ca^{2+} deprivation and verapamil on the tension increases of the golden hamster tracheal strip-chain preparations caused by substance P (0·3–10 μM), carbachol (0·01–10 μM) and KCl (20–60 mM). Agonists were applied cumulatively to the organ bath containing Krebs solution at 37 °C. The ordinate scale represents the response as a % of the maximum response caused by carbachol. The symbols represent the initial concentration-response curve (●) and second concentration-response curve in the presence of verapamil (1 μM, ○) or in Ca^{2+}-free, EGTA (0·1 mM) containing Krebs solution (□). Rest intervals between curves were 30–40 min. Each point is the mean value from at least 6 experiments and vertical bars represent s.e.m. $^{*}P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$; $^{***}P < 0{\cdot}001$ when compared with the values of the initial concentration-response curve (unpaired *t*-test).

pamil, indicating that these contractions are partly mediated by the mobilization of intracellular Ca^{2+}. Therefore, it seems reasonable to assume that cooling-induced augmentation of the response to substance P may also be due to the acceleration of Ca^{2+} release from intracellular storage sites. In fact, caffeine, which is known to release intracellular Ca^{2+} (Saida 1981, 1982; Endo et al 1982), markedly contracted the tracheal muscle from the golden hamster at 20 °C but not at 37 °C.

In conclusion, cooling may augment the contractile response of the golden hamster tracheal muscle to substance P by accelerating Ca^{2+} release from intracellular storage sites. This may give a new aspect to the understanding of airway disorders, such as exercise-induced asthma. That is, it is speculated that heat loss during exercise will induce bronchoconstriction by increasing the responsiveness of airway smooth muscle to substance P.

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