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Effect of vanadate on cooling-induced tension changes in K-depolarized smooth muscles from guinea-pigs

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Sodium vanadate reversed cooling-induced relaxation of K-depolarized taenia coli of guinea-pigs but failed to reverse it in the portal vein and uterus. It potentiated cooling-induced contraction of K-depolarized vas deferens and ureter. These effects were not mediated by the inhibition of Na,K-ATPase, but by the inhibition of the Ca-pump.

It is generally accepted that high K-induced contraction of smooth muscles is initiated by an influx of Ca from the extracellular space (Bolton 1979; Van Breemen et al 1979) and that the tonic component of the contracture is maintained by the equilibration of Ca influx and extrusion (Sunano 1976; Shimodan & Sunano 1981). Therefore, treatments which affect the Ca pumps of the cell membrane or sarcoplasmic reticulum would be expected to influence high K-induced tonic contraction. Low temperature, for example, has been known to depress the Ca pumps of the membranous systems of smooth muscles (Hurwitz et al 1975; Deth 1978; Droogmans & Casteels 1981; Ueno 1985) and to induce tension development in K-depolarized vas deferens (Sunano 1981).

Vanadate, which is known as a potent inhibitor of Na,K-ATPase, also inhibits Ca-ATPase or Ca-uptake by the membranous systems of smooth muscles (Wibo et al 1981; Popescu & Ignat 1983; Raeymaekers et al 1983) and induces tension development in depolarized vas deferens (Sunano et al 1985). In K-depolarized taenia coli, on the other hand, cooling causes relaxation. Vanadate has also been reported to induce relaxation in K-depolarized taenia coli on prolonged application (Ueda et al 1982). However, the differences in the mechanisms of cooling and of vanadate among smooth muscles have not been well clarified. In the present experiments, the effects of sodium vanadate on cooling-induced relaxation of K-depolarized preparations from various smooth muscles of the guinea-pig have been studied.

Materials and methods

Taenia coli, vasa deferentia, ureters, portal veins and uteri were dissected from guinea-pigs, 400–600 g. Longitudinal preparations of these tissues were incubated in a modified Tyrode solution of the following composition kept at 35 °C: (mM) NaCl, 137; KCl, 2·7; $CaCl_2$, 2·0; $MgCl_2$, 1·0; $NaHCO_3$, 11·9; NaH_2PO_4, 0·4; glucose, 5·6 and equilibrated with a mixture of 95% O_2 and 5% CO_2. K-Tyrode solution was made by replacing all NaCl in the modified Tyrode solution with KCl, and K-60 mM Tyrode solution was prepared by mixing the modified Tyrode solution and K-Tyrode solution in the appropriate ratio.

The temperature of the incubation medium was controlled by circulating water of the desired temperature in the outer chamber of an organ bath, and the change in temperature was monitored by using a thermistor probe in the bath. Changes in pH of the incubation medium due to the lowering of the temperature were not corrected, since similar changes produced no significant alteration of tonic tension by high-K.

The preparations were first subjected to high-K-induced contracture by changing the incubation medium from Tyrode to K-Tyrode or K-60 mM-Tyrode solution. Cooling experiments were performed after the tonic component of high-K-induced contracture became stable, i.e. at least 15 min after the application of high-K Tyrode solution, since the effects were reproducible after this time and almost the same responses could be observed as long as the tonic component was sustained without obvious change in tension. The cooling treatment was applied by switching the circulating water from 35 °C to 20 °C or 15 °C, since the highest contractile responses of the vas deferens and ureter were observed at these temperatures (Sunano 1981; Sunano & Miyazaki 1981). The duration of cooling was varied between 10 and 30 minutes so that the maximum change in tension could be observed.

Results

Preparations from the vas deferens and ureter showed a contracture composed of the phasic and tonic components in response to the change of incubation medium from Tyrode to high-K Tyrode solution. The tonic contraction was sustained for longer than 60 min without obvious reduction in tension. Cooling applied during the course of the tonic component caused an elevation of tension. The effects could be obtained repeatedly without marked alteration, as has previously been reported (Sunano & Miyazaki 1981).

Sodium vanadate (2 mM) applied during the course of the tonic component of high K-induced contracture induced sustained tension development. The heights of the developed tension in the vas deferens were 226 ± 22·1% (mean ± s.e., n = 6) in K-Tyrode solution and

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332 ± 43·7% (mean ± s.e., n = 6) in K-60 mM Tyrode solution, respectively, of the heights of the tonic component of high K-induced contracture. The heights of the developed tension in the ureter were 193 ± 11·8% (mean ± s.e., n = 6) in K-Tyrode solution and 1133 ± 53·3% (mean ± s.e., n = 6) in K-60 mM Tyrode solution of the heights of the high-K-induced tonic contraction.

Cooling-induced development of tension was markedly potentiated in the presence of sodium vanadate both in the vas deferens and ureter (Fig. 1, Table 1). In

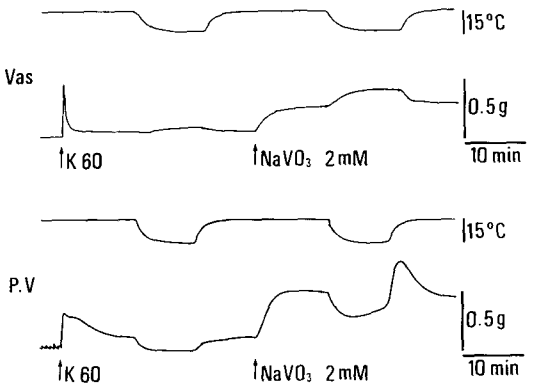

FIG. 1. Effects of cooling-induced contraction in the vas deferens and of cooling-induced relaxation in the portal vein. The temperature was lowered from 35 to 15 °C as indicated. K60 and $NaVO_3$ indicate the application of K-60 mM Tyrode solution and sodium vanadate (2 mM), respectively. Note the differences in cooling-induced responses. The average tension changes by cooling in vas deferens, ureter, portal vein and taenia coli in K-Tyrode solution were 0·39 ± 0·05 g, 0·16 ± 0·03 g, −0·08 ± 0·03 g, and −1·25 ± 0·24 g, and those in K-60 mM Tyrode solution were 0·45 ± 0·06 g, 0·41 ± 0·06 g, −0·22 ± 0·06 g and −0·70 ± 0·12 g respectively (mean ± s.e., n = 6 to 14).

Table 1. Relative amplitudes of cooling-induced contractions and relaxations.

	Ureter	Vas Def.	Portal V.	Taenia
K-T	+100	+100	−100	−100
K-T $NaVO_3$	+300 ± 6·4 (n = 28)	+140 ± 6·6 (n = 12)	−206 ± 21·6 (n = 7)	+60 ± 18·0 (n = 6)
K60	+100	+100	−100	−100
K60 $NaVO_3$	+256 ± 12·1 (n = 25)	+195 ± 21·9 (n = 6)	−175 ± 13·1 (n = 6)	+166 ± 41·1 (n = 9)

Control contractions and relaxations were taken as +100 and −100, respectively, and contractions and relaxations in the presence of 2 mM sodium vanadate ($NaVO_3$) were expressed as relative values of control height (mean ± s.e.). K-T, K-Tyrode solution. K60, K-60 mM Tyrode solution.

the vas deferens, sodium vanadate at (2 mM) also induced development of tension in the polarized preparation and often initiated spontaneous twitch-like contractions. Cooling caused cessation of the spontaneous contractions but had no obvious effect on the elevated basal tension, i.e. no tension developed as a result of the cooling treatment. Sodium vanadate could also induce tension development in the K-depolarized preparation in the absence of Ca. The increase in the tension developed by 2 mM sodium vanadate observed 60 min after Ca removal was 46 ± 3·1% (mean ± s.e., n = 5) of that observed in the presence of Ca. Cooling to 15 °C caused a 38 ± 4·2% (mean ± s.e., n = 5) relaxation of the tension induced by sodium vanadate in the absence of Ca. Ouabain at concentrations up to 10^{-5} M, showed no obvious effect on the tonic component of K-contracture both in the vas deferens and ureter. The development of cooling-induced tension was not greatly affected by 10^{-5} M ouabain either in the vas deferens or ureter, nor were the effects of sodium vanadate on the development of cooling-induced tension blocked by treatment with ouabain of 10^{-5} M.

Preparations from the taenia coli and portal vein showed contracture that was also composed of the phasic and tonic components in response to the application of high-K solution; the tonic component was sustained for at least 60 min without obvious reduction in tension. In these preparations, cooling treatment applied during the course of the tonic component of high-K-induced contracture caused a relaxation (Fig. 1, Table 1). The relaxation of these preparations by cooling was also reproducible and similar responses could be observed as long as the tonic contraction was sustained without obvious change in tension.

Sodium vanadate (2 mM) induced development of tension in the portal vein which was 239 ± 18·2% (mean ± s.e., n = 7) of the height of the tonic component of the contracture induced by K-60 mM Tyrode solution. Cooling treatment applied in the presence of sodium vanadate induced a deeper relaxation than that in the absence of the drug, though slight re-elevation of tension was observed when cooling treatment was prolonged (Fig. 1). By rewarming the preparation, a phasic rebound contraction was induced. Similar results to those of the portal vein could be obtained in the preparations from uterus.

In the taenia coli, sodium vanadate, 2 mM, induced a small phasic contraction followed by slow relaxation to the level below the height of high-K-induced tonic contraction. In the present experiments, the cooling treatment was applied at the time when the tension had returned to the level before the application of sodium vanadate, so that further relaxation could be observed. The cooling treatment, however, caused a marked tension development in the presence of sodium vanadate (Fig. 2a, Table 1). Treatment with ouabain (5×10^{-6} M) caused a slow relaxation without inducing a phasic contraction, but could not reverse the cooling-induced relaxation (Fig. 2b). It also failed to block the reversing effect of sodium vanadate on cooling-induced relaxation. An increase in Ca concentration, which has been known to potentiate the cooling-induced contraction in the vas deferens markedly (Sunano 1981; Sunano & Miyazaki 1981), also failed to reverse the cooling-

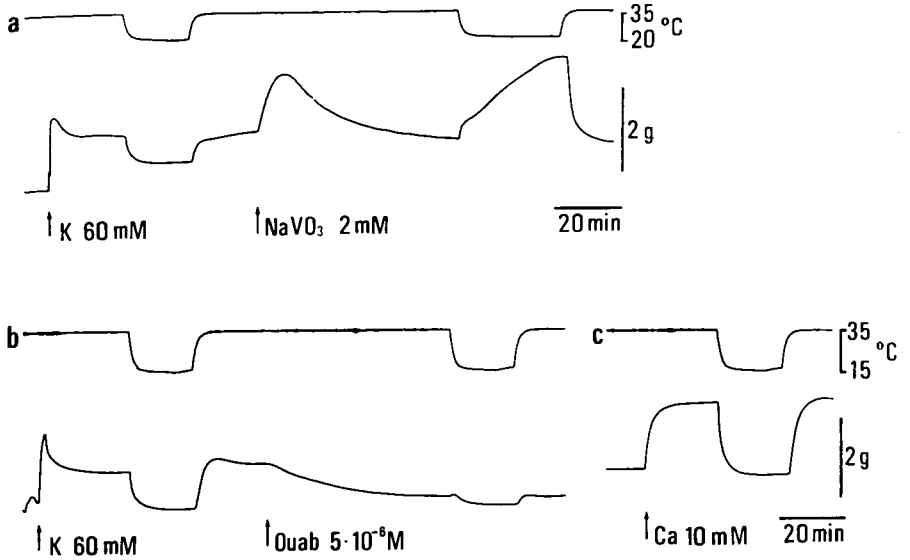

FIG. 2. Effects of sodium vanadate, ouabain and increased Ca concentration on cooling-induced relaxation of K-depolarized taenia coli. The temperature was reduced from 35 to 20 °C in a and to 15 °C in b and c as indicated. Ouab and Ca 10 mM indicate the application of ouabain (5×10^{-6} M) and increase of Ca concentration from the control (2 mM) to 10 mM, respectively. Other explanations are the same as in Fig. 1b and c were taken from the same preparation, and c was taken 45 min after the application of K-60 mM Tyrode solution.

induced relaxation of the taenia coli, though it caused an elevation of the tension of the tonic contraction of high-K-induced contracture (Fig. 2c).

Discussion

In the vas deferens and ureter, cooling treatment of depolarized preparations induced development of tension. Since the tension developments were abolished in the absence of extracellular Ca or in the presence of Ca antagonists (Sunano 1981, 1984; Sunano & Miyazaki 1981), it is assumed that the tension development would be related to an influx of Ca brought about by depolarization of the membrane (Sunano 1976; Shimodan & Sunano 1981). The cooling treatment is known to inhibit the extrusion of Ca taken up (Deth 1978; Droogmans & Casteels 1981) and thus leads to the increase in intracellular Ca. Vanadate inhibits Ca-ATPase or Ca uptake by the membranous systems of smooth muscles (Wibo et al 1981; Popescu & Ignat 1983; Raeymaekers et al 1983). The enhancement of the cooling-induced tension development by vanadate, therefore, can be explained by an exaggerated inhibition of Ca sequestration in the depolarized preparations. Similarly, the reversal of the cooling-induced relaxation observed in the taenia coli may be explained by inhibition of the sequestration of the influxed Ca to overcome the reduction of Ca influx (Krejci & Daniel 1970; Droogmans & Casteels 1981) or of ATPase of the actomyosin system (Murphy 1971) at low temperature.

Although the basal tension was different in the presence and absence of sodium vanadate, this might not be the cause of the potentiation of cooling-induced tension development, since it has been shown that the level of the basal tension does not play an important role in determining the amplitude of the cooling-induced contraction (Sunano 1981). In addition, it was demonstrated in the present experiments that the reversal of cooling-induced relaxation by vanadate was not dependent on the change in pre-existing tension. Since the changes in cooling-induced tension were reproducible as long as the tonic contraction was sustained without obvious changes (present experiments and Sunano & Miyazaki 1981), the difference in time after application of high K solution might not be the cause of the alteration of the cooling-induced tension changes.

The inability of vanadate to reverse the cooling-induced relaxation in portal vein remains unexplained. However, the present results suggest that the Ca sequestering activities of membranous systems and their sensitivity to cooling and to vanadate might vary among the types of smooth muscle. Although the cooling-induced relaxation of the portal vein seemed to be greater in the presence of sodium vanadate, this may be due to higher pre-existing tension; the cooling-induced relaxation was almost the same as the height of high K-induced tonic contraction.

In summary, sodium vanadate potentiated the cooling-induced contraction of the depolarized preparations of the vas deferens and ureter, and reversed the cooling-induced relaxation of a taenia coli, presumably

by inhibiting the Ca sequestering activities of the membranous systems, but it failed to reverse the cooling-induced relaxation of the portal vein. The difference in Ca sequestering activities may be the cause of the differences in the action of sodium vanadate.

REFERENCES

Bolton, T. B. (1979) Physiol. Rev. 59: 606–718
Deth, R. C. (1978) Am. J. Physiol. 234: C139–C145
Droogmans, G., Casteels, R. (1981) Pflügers Arch. 391: 183–189
Hurwitz, L., Debbas, G., Little, S. (1975) Mol. Cell. Biochem. 8: 31–41
Krejci, I., Daniel, E. E. (1970) Am. J. Physiol. 219: 263–269
Murphy, R. A. (1971) Ibid. 220: 1494–1500
Popescu, L. M., Ignat, P. (1983) Cell Calcium 4: 219–235
Raeymaekers, L., Wytack, F., Eggermont, J., Deschutter, G., Casteels, R. (1983) Biochem. J. 210: 315–322
Shimodan, M., Sunano, S. (1981) Jap. J. Physiol. 31: 15–27
Sunano, S. (1976) Ibid. 26: 717–727
Sunano, S. (1981) Experientia 37: 1165–1166
Sunano, S. (1984) Jap. J. Pharmacol. 34: 51–56
Sunano, S., Miyazaki, E. (1981) Jap. J. Smooth Muscle Res. 17: 27–33
Sunano, S., Shimada, T., Shimamura, K. (1985) Ibid. 21: 95–105
Ueda, F., Kishimoto, T., Ozaki, H., Urakawa, N. (1982) Jap. J. Pharmacol. 32: 149–157
Ueno, H. (1985) J. Physiol. (Lond.) 363: 103–117
Van Breemen, C., Aaronson, P., Loutzehieser, R. (1978) Pharmacol. Rev. 30: 167–208
Wibo, M., Morel, N., Godfraind, T. (1981) Biochem. Biophys. Acta 649: 651–660

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Diazepam facilitates reflex bradycardia in conscious rats

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The effects of diazepam on cardiovascular function were assessed in conscious rats. Intravenous administration of diazepam (1–30 mg kg^{-1}) produced a dose-dependent decrease in both the mean arterial pressure and the heart rate. Also, reflex bradycardia was produced in rats by intravenous infusion of adrenaline (1·25–2·5 μg kg^{-1}). Intravenous pretreatment of the rats with diazepam, although causing no change in the adrenaline-induced pressor effect, did enhance the adrenaline-induced reflex bradycardia. However, the diazepam enhancement of adrenaline-induced reflex bradycardia was antagonized by pretreatment of rats with an intravenous dose of picrotoxin (an agent blocks chloride channels by binding to sites associated with the benzodiazepine-GABA-chloride channel macromolecular complex). The data indicate that diazepam acts through the benzodiazepine-GABA-chloride channel macromolecular complex within the central nervous system to facilitate reflex bradycardia mediated through baroreceptor reflexes in response to an acute increase in arterial pressure.

Benzodiazepines represent a large class of compounds widely used in clinical practice for the treatment of anxiety, insomnia, convulsions and muscular rigidity (Garattini et al 1973; Greenblatt & Shader 1974; Dantzer 1977). Considerable attention has been given to their cardiovascular effects. For example, diazepam, in an intravenous dose of 60 mg, caused a decrease in respiration, blood pressure, left ventricular stroke work and cardiac output (Rao et al 1973). However, to our knowledge, relatively little information is available about the effects of diazepam on reflex bradycardia. The arterial baroreflex system is regarded as one of the most powerful and rapidly acting homeostatic mechanisms for regulating blood pressure (Korner 1971; Kirchheim 1976). Therefore, in the present study, the effects of systemic administration of diazepam or picrotoxin on the reflex bradycardia produced in response to elevation of arterial pressure induced by an intravenous infusion of adrenaline were investigated in conscious rats.

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Materials and methods

Male Sprague-Dawley rats, 250–300 g, were housed individually in wire mesh cages in a room maintained at 22 ± 1·0 °C. They were given free access to tap water and granular chicken feed supplied by Taiwan Sugar Corporation. The animals were anaesthetized with ether. The femoral artery and the femoral vein were catheterized, for blood pressure measurement and intravenous infusion, respectively. Diazepam (1–30 mg kg^{-1}), picrotoxin (0·1–0·3 mg kg^{-1}) or 0·9% NaCl (saline) was administered into the femoral vein. Either the drug or vehicle was administered at a volume of 1·5 mL kg^{-1}. The reflex bradycardia was induced by intravenous infusion of adrenaline (epinephrine, USP, Retired Servicemen's Pharmaceutical Plant of Taiwan) in conscious animals (Lin & Chern 1979; Lin et al 1980). The blood pressure, recorded from the femoral artery, was monitored with a Statham P23Ac transducer, and the heart rate was monitored with a Grass 7C tachometer, triggered by arterial pulses. All recordings were made on a four-channel Grass 7C polygraph.