EFFECTS OF NONSPECIFIC SMOOTH MUSCLE RELAXANTS ON TISSUE CONCENTRATIONS OF HIGH ENERGY PHOSPHATES AND MECHANICAL ACTIVITY OF NORMAL POLARIZED AND DEPOLARIZED INTESTINAL SMOOTH MUSCLES FROM GUINEA PIG

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Abstract—Effects of nonspecific smooth muscle relaxants, an uncoupler and removal of Ca ions from physiological solution on the tissue concentrations of high energy phosphates, such as ATP and creatine phosphate (CP) and tension of the normal polarized and KCI-depolarized intestinal smooth muscles of guinea pig were studied. Decrease of CP-concentration induced by dinitrophenol (DNP; 10^{-4} M) was accompanied by relaxation of the normal polarized and KCI-depolarized smooth muscles. DNP slightly (but significantly) decreased ATP-concentration in the normal polarized and KCI-depolarized smooth muscles. Application of papaverine (3 \times 10^{-5} M) relaxed the normal polarized taenia immediately but increased CP-concentration at 5 and 10 min and decreased the concentration at 20 min. When the depolarized smooth muscle was considerably relaxed by papaverine (3 \times 10^{-5} M), there was little influence on the CP-concentration. After relaxation of the depolarized taenia as induced by papaverine had reached a maximal amplitude, CP-concentration decreased significantly. ATP-concentration was little influenced by papaverine in the normal polarized and KCI-depolarized muscles. Although the treatments with a synthetic antispasmodic drug, Aspaminol (3 \times 10^{-4} M), which was found to inhibit Ca-uptake by the intestinal smooth muscles, a Ca-blocker, D-600 (10^{-6} M) and removal of Ca ions from physiological solution relaxed the polarized and depolarized smooth muscles, the tissue concentrations of CP and ATP increased. These phenomena are considered to be due to decrease of the intracellular Ca-concentration.

After papaverine was shown to have a potent inhibitory action on cyclic AMP-phosphodiesterase activity (1), it was reported by Takayanagi et al. (2) that the cyclic AMP levels increased significantly in the guinea pig taenia caecum relaxed by papaverine. Increase in the tissue level of cyclic AMP is considered to be a possible mechanism of the smooth muscle relaxation induced by papaverine. Furthermore, Takayanagi et al. (3) indicated that relaxation of the KCI-depolarized taenia caecum of guinea pig induced by papaverine and some smooth muscle relaxants was mainly due to inhibition of Ca-uptake. On the other hand, some authors (4–6) reported that papaverine inhibited mitochondrial respiration in in vivo experiments. Therefore, the smooth muscle relaxing effects of papaverine are
attributed to different mechanisms. To investigate the extent to which metabolic inhibition plays a role in the relaxation of smooth muscle induced by papaverine and other smooth muscle relaxants, we measured ATP- and creatine phosphate (CP)-concentrations of normal polarized and KCl-depolarized smooth muscles after treatment with smooth muscle relaxants, an uncoupler, and Ca-free bath fluid. The smooth muscle tissue relaxed when these treatments were given.

**METHODS AND MATERIALS**

Male guinea pig, weighing 300 to 400 g, were sacrificed by a blow on the head. A piece (3-4 cm) of taenia was isolated from the caecum and suspended in a 30 ml organ bath filled with normal physiological solution (NaCl 154, KCl 5.6, CaCl₂ 2.2, MgCl₂ 2.1, NaHCO₃ 5.9 and glucose 2.8 mM) kept at 32°C and bubbled with air. Mechanical responses were isometrically recorded under a resting tension of 2 g. Tension related to the maximal relaxation was determined by immersing the taenia in physiological solution (omitting CaCl₂ from the normal physiological solution), and the the taenia was then washed and suspended in normal physiological solution for 60 min. To study the effects of papaverine and nonspecific smooth muscle relaxants on ATP- and CP-concentrations in depolarized taenia caecum of guinea pig, we used isotonic 159.6 mM KCl-physiological solution which was made by replacing all of NaCl and NaHCO₃ in normal Locke Ringer solution by KCl and KHCO₃. Therefore, in some experiments the smooth muscles were treated with isotonic 159.6 mM KCl-physiological solution for 30 min. The normal polarized and depolarized preparations were frozed in liquid nitrogen immediately after the mechanical response to a drug had been observed. These frozen preparations were then homogenized in 0.5 ml of 3% perchloric acid in an ice-bath. The homogenate was centrifuged for 15 min at 13,000 × g at 0°C and the supernatant was neutralized with 2 N KOH, using bromothymol blue as an external pH-indicatior (7). ATP- and CP-concentrations were estimated by the method of Lowry et al. (8). Protein was assayed by the method of Lowry et al. (9), using bovine serum albumin as the standard. The drug concentrations used were sufficient to induce a maximal relaxation of smooth muscle. ATP- and CP-concentrations are expressed as percents of ATP-concentration at 0 in the abscissa of the figures. ATP- and CP-concentrations in Figs. 1 to 4 are expressed as percents of ATP-concentration after 60 min incubation of the taenia caecum in normal physiological solution. On the other hand, ATP- and CP-concentrations in Figs. 5 to 7 are expressed as percents of ATP-concentration in the taenia caecum immersed in isotonic 159.6 mM KCl physiological solution for 30 min. Decrease of tension after the treatment of the smooth muscles with a test drug was expressed as a percent of the maximum response (maximally decreased tension) obtained by replacement of normal physiological solution with Ca-free physiological solution. Therefore, spontaneous tension in normal physiological solution was expressed as 100 percent in all the figures. All the values are expressed as means with S.E. of 6 experiments.

_Drug used:_ 2,4-dinitrophenol (DNP: Wako-Junyaku, Japan), papaverine hydrochloride (Daiichi Kagaku, Japan), Aspaminol (1,1-diphenyl-3-piperidinobutanol hydrochloride;
Kowa, Japan), D-600 (Knoll) and glycoletherdiamine tetraacetic acid (GEDTA; Wako-Junyaku, Japan).

RESULTS

The tissue concentrations of ATP and CP estimated immediately after dissection of the taenia from caecum were 13.20 ± 0.66 and 12.04 ± 1.36 n moles/mg protein (mean ± S.E. of 4 experiments), respectively. The tissue concentrations after the 30 and 60 min incubations in normal physiological solution kept at 32°C and bubbled with air were 12.33 ± 0.71 and 11.61 ± 0.48 n moles/mg protein for ATP-concentrations, and 12.36 ± 1.25 and 12.89 ± 1.33 n moles/mg protein. The values are presented as mean ± S.E. of 6 experiments. These results suggest that ATP- and CP-concentrations in the taenia caecum after 30 and 60 min incubations with normal physiological solution were not significantly different from concentrations in the preparations immediately after dissection of the taenia from the caecum. Ten min after addition of DNP (10^{-1} M), tissue concentration of CP reached a minimum but smooth muscle relaxation was observed to the extent of 50 to 60 percent of the maximum amplitude (Fig. 1). Though the results on ATP-concentration were not clearcut, ATP-concentration reached the minimum level before mechanical response to DNP (10^{-1} M) developed maximally (Fig. 1). Application of papaverine (3 × 10^{-5} M) relaxed the smooth muscle immediately (Fig. 2). However, CP-concentration increased 5 and 10 min after

Fig. 1. Effects of DNP (10^{-4} M) on tension and ATP- and CP-concentrations of guinea pig taenia caecum suspended in normal physiological solution. After 60 min incubation in normal physiological solution, the bath fluid was replaced by the solution containing DNP at 0 min. ---: tension, --- ATP, --- CP. No. of experiments and the methods of expressing tension and ATP- and CP-concentrations were as described in Materials and methods.

* significant difference from the control value (at 0 min) at p < 0.05.
application of papaverine (3 x 10^{-5} M) and decreased at 20 min (Fig. 2). Aspaminol (3 x 10^{-4} M), a synthetic antispasmodic drug, which has little inhibitory action on cyclic AMP-phosphodiesterase activity (3) increased CP-concentration and decreased tension of the normal polarized taenia (Fig. 2). As was the case with Aspaminol, the treatments with a

![Graph](image)

**Fig. 2.** Effects of papaverine (3 x 10^{-5} M) and Aspaminol (3 x 10^{-4} M) on tension and ATP- and CP-concentrations of the normal polarized taenia caecum. After 60 min incubation in normal physiological solution, the drugs were added at 0 min. —: tension, —: ATP, —: C.P. No. of experiments and the methods of expressing tension and ATP- and CP-concentrations were described in Materials and methods. * Significant difference from the control value (at 0 min) at p<0.05.

![Graph](image)

**Fig. 3.** Effects of the treatments of the normal polarized taenia caecum with D-600 and with Ca-free physiological solution containing GEDTA (0.2 mM) on tension and ATP- and CP-concentrations. After 60 min incubation in normal physiological solution, D-600 (10^{-6} M) was added or the bath fluid was replaced by Ca-free physiological solution containing GEDTA (0.2 mM) at 0 min. —: tension, —: ATP, —: C.P. No. of experiments and the methods of expressing tension and ATP- and CP-concentrations were as described in Materials and methods. * Significant difference from the control value (at 0 min) at p<0.05.
Ca-blocker, D-600 (10^{-6} M) and with Ca-free physiological solution containing GEDTA (0.2 mM) increased CP-concentration, while both treatments decreased tension of the normal polarized taenia (Fig. 3). ATP-concentration in the normal polarized taenia was

Fig. 4. Effect of isotonic 159.6 mM KCl physiological solution on tension and ATP- and CP-concentrations of guinea pig taenia caecum. After 60 min incubation in normal physiological solution, the bath fluid was replaced by isotonic 159.6 mM KCl physiological solution at 0 min. ---: tension, -----: ATP, ---: C.P. No. of experiments and the methods of expressing tension and ATP- and CP-concentrations were as described in Materials and methods. * Significant difference from the control value (at 0 min) at p<0.05.

Fig. 5. Effect of DNP (10^{-4} M) on tension and ATP- and CP-concentrations of the depolarized taenia caecum of guinea pig. After 60 min incubation with normal physiological solution and 30 min treatment with isotonic 159.6 mM KCl physiological solution, the bath fluid was replaced by isotonic 159.6 mM KCl solution containing DNP (10^{-4} M) at 0 min. ---: tension, -----: ATP, ---: C.P. No. of experiments and the methods of expressing tension and ATP- and CP-concentrations were described in Materials and methods. * Significant difference from the control value (at 0 min) at p<0.05.
little influenced by the treatments with papaverine but increased significantly by Aspaminol, D-600 and Ca-free physiological solution (Figs. 2, 3).

**Fig. 6.** Effects of papaverine (3 x 10^{-5} M) and Aspaminol (3 x 10^{-4} M) on tension and ATP- and CP-concentrations of the depolarized taenia caecum of guinea pig. After 60 min incubation in normal physiological solution and 30 min treatment with isotonic 159.6 mM KCl-physiological solution, the drugs were added at 0 min. –●–: tension, –■–: ATP, –○–: C.P. No. of experiments and the methods of expressing tension and ATP- and CP-concentrations were as described in Materials and Methods. * Significant difference from the control value (at 0 min) at p<0.05.

**Fig. 7.** Effects of D-600 (10^{-6} M) and removal of Ca^{2+} from bath fluid on tension and ATP- and CP-concentrations of the depolarized taenia caecum of guinea pig. After 60 min incubation in normal physiological solution and 30 min treatment with isotonic 159.6 mM KCl solution, D-600 was added or the bath fluid was replaced by Ca-free 159.6 mM solution (omitting CaCl_{2} from isotonic 159.6 mM KCl-solution) and addition of GEDTA (0.2 mM), at 0 min. –●–: tension, –■–: ATP, –○–: C.P. No. of experiments and the methods of expressing tension and ATP- and CP-concentrations were as described in Materials and Methods. * Significant difference from the control value (at 0 min) at p<0.05.
Though replacement of normal physiological solution with isotonic 159.6 mM KCl-physiological solution induced a phasic contraction followed by a tonic contraction, CP-concentration decreased after replacement with isotonic KCl-physiological solution (Fig. 4). In the KCl-depolarized muscles, DNP (10^{-4} M) caused the decrease in ATP- and CP-concentrations followed by tension decline (Fig. 5). While the KCl-depolarized smooth muscle relaxed about 60 percent of the maximum amplitude 10 min after application of papaverine (3 \times 10^{-5} M), a significant decrease in CP-concentration of the same preparation was observed at 20 but not 10 min after the treatment (Fig. 6). ATP-concentration was little influenced by papaverine (Fig. 6).

As observed in the normal polarized smooth muscles, the treatments with Aspaminol (3 \times 10^{-4} M), D-600 (10^{-5} M) and with Ca-free isotonic 159.6 mM KCl-physiological solution, (omitting CaCl_2 from the isotonic 159.6 mM physiological solution and addition of GEDTA (0.2 mM)), increased CP-concentration considerably and ATP-concentration slightly but significantly, though these treatments decreased tension of the KCl-depolarized taenia caecum (Figs. 6, 7).

**DISCUSSION**

We found that the tissue concentrations of ATP and CP were not influenced by the 30 and 60 min incubation in normal physiological solution. Bueding et al. (7) reported that incubation of tissues with Krebs solution, which contained glucose 11.5 mM kept at 37°C and bubbled with a mixture of 97% oxygen and 3% carbon dioxide increased ATP- and CP-concentrations. Discrepancies between our results and the findings of Bueding et al. (7) may be attributed to differences in experimental conditions such as temperature and glucose-and oxygen-concentrations in bath fluid.

Onset of relaxation of KCl-depolarized smooth muscle induced by nonspecific smooth muscle relaxants was generally slow. Therefore, KCl-depolarized muscle is considered to be suitable for testing the correlation between the tissue concentrations of high energy phosphates and smooth muscle relaxation, as induced by drugs. We used the normal polarized and KCl-depolarized smooth muscles, however, changes in CP-concentration in the normal polarized muscle after application of papaverine or Aspaminol were different from those in the KCl-depolarized muscle. We have no explanation for these differences.

DNP (10^{-4} M) decreased ATP- and CP-concentrations in the polarized and KCl-depolarized smooth muscles. These results were accompanied by a decline in tension. Born and Bülbring (10) also reported that in higher concentrations of DNP, ATP-concentration decreased with the decrease in tension. These results suggest that the tension decline induced by DNP is due to a decrease energy supply. Relaxation of the normal polarized or KCl-depolarized muscle induced by papaverine was not preceded by a decrease in the ATP- and CP-concentrations. These findings suggest that decrease in the tissue concentrations of energy rich phosphates does not initiate the relaxation of smooth muscle, as induced by papaverine. It is, however, probable that metabolic inhibition of papaverine potentiates the relaxation of smooth muscle by papaverine. In the normal polarized and
depolarized smooth muscles, treatment with Aspaminol, which reportedly inhibits Ca-uptake (11), and D-600, a Ca-blocker, increased ATP- and CP-concentrations with tension decline. As similar results were obtained by omission of Ca\(^{2+}\) from the physiological solution, increase in the CP- and ATP-concentrations is probably due to a decrease in intracellular Ca\(^{2+}\)-concentration. Nishiki et al. (12) reported that in rat heart muscles, a fall in intracellular Ca\(^{2+}\)-concentration decreases mechanical activity and hyperactivity of respiration and leads to a remarkable increase in ATP- and CP-concentrations. Our findings on the ATP- and CP-concentrations in the smooth muscles were similar.

Changes in ATP-concentrations after the treatment of smooth muscles were greater than the changes seen with CP-concentration. Thus, ATP-concentration in the smooth muscle may be replenished by activation of creatine kinase (13). Effects of these treatments on the phosphorylase activity have been reported elsewhere (14).

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