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EFFECTS OF HYDRALAZINE AND VERAPAMIL ON PHOSPHORYLASE ACTIVITY AND GUANOSINE CYCLIC 3',5'-MONOPHOSPHATE LEVELS IN GUINEA-PIG TAENIA COLI

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1 The roles of guanosine cyclic 3',5'-monophosphate (cyclic GMP) and calcium in the relaxation produced by hydralazine and verapamil in potassium-depolarized guinea-pig taenia coli have been investigated.

2 Depolarization of isolated strips of guinea-pig taenia coli by 124 mM KCl caused sustained contractures and increases in tissue levels of cyclic GMP.

3 The KCl-induced increases in cyclic GMP levels appeared to be calcium dependent. No increases in cyclic GMP levels were seen in strips of taenia coli depolarized in the absence of calcium. Re-addition of calcium to the depolarizing solution contracted the muscles and increased cyclic GMP levels. When calcium was removed from the depolarizing solution during the sustained, tonic phase of a KCl-induced contracture, both tension and cyclic GMP levels returned to control values.

4 Administration of 50 μM verapamil to KCl-contracted muscles completely relaxed the muscles and caused cyclic GMP levels to return to control values within 14 min. Hydralazine, 2 mM, on the other hand, relaxed the depolarized muscles without lowering cyclic GMP levels. No significant changes in cyclic AMP levels were seen in any of these experiments.

5 Similar results were obtained in an analogous series of experiments in which glycogen phosphorylase activity was measured instead of cyclic GMP levels. Activation of phosphorylase during contractions of guinea-pig taenia coli had previously been reported to be a calcium-dependent phenomenon.

6 It was concluded that increases in tissue levels of cyclic GMP (or of cyclic AMP) were not responsible for the relaxant effects of hydralazine or verapamil in these experiments. It was also suggested, based on our results, that verapamil exerted its relaxant effects in the depolarized taenia coli by lowering cytoplasmic calcium levels, whereas hydralazine relaxed the depolarized muscles without lowering intracellular calcium activity.

Introduction

It has recently been suggested that increases in guanosine cyclic 3',5'-monophosphate (cyclic GMP) levels may be responsible for the smooth muscle relaxant effects of a variety of drugs including sodium nitroprusside, hydralazine and verapamil (Schultz, Schultz & Schultz, 1977). This suggestion is supported by the observations that various relaxant drugs can increase cyclic GMP levels in smooth muscle preparations (Diamond & Blisard, 1976; Schultz *et al.*, 1977; Katsuki, Arnold & Murad, 1977) and that exogenously added cyclic GMP and its derivatives are capable of relaxing isolated smooth muscles (Katsuki & Murad, 1977; Schultz *et al.*, 1977). Some preliminary evidence against this hypothesis has been presented in a recent brief communication (Diamond & Janis, 1978). In the latter study it was reported that cyclic GMP levels were elevated during KCl-induced contracture of isolated vas deferens of the rat and that hydralazine and verapamil were capable of relaxing these depolarized muscles with no further increases in cyclic GMP levels beyond those caused by the KCl alone. Thus, under these conditions, increases in cyclic GMP levels did not appear to be responsible for the relaxant effects of hydralazine or verapamil. The present paper describes similar experiments in another type of smooth muscle, the guinea-pig taenia coli. As was the case with vas deferens, the results in taenia coli do not support the hypothesis that increases in cyclic GMP levels are responsible for the smooth muscle relaxant effects of hydralazine or verapamil.

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In addition, the effects of the relaxant drugs and calcium removal on levels of cyclic GMP and glycogen phosphorylase in KCl-contracted muscles were compared. The results suggest that relaxation of depolarized taenia coli by verapamil is accompanied by a decrease in intracellular calcium concentration. Hydralazine, on the other hand, may relax the muscles without lowering intracellular calcium activity.

Methods

Strips of taenia coli were removed, under ether anaesthesia, from female guinea-pigs weighing 300 to 400 g. The strips were suspended in isolated organ baths at 37°C for recording of isometric tension as previously described (Diamond & Brody, 1966). At least one control strip, in addition to one or more test strips, was obtained from each guinea-pig, so that control and drug-treated strips were obtained from the same population of animals. All muscles were equilibrated for 45 to 60 min (at a resting tension of approx. 0.3 g) in a physiological salt solution with the following composition (mM): NaCl 118, KCl 5.7, $MgSO_4$ 2.37, $CaCl_2$ 1.26, NaH_2PO_4 1.17, $NaHCO_3$ 25 and glucose 11. The muscles were oxygenated by bubbling with a mixture of 95% O_2 and 5% CO_2 which maintained the pH of the solutions at approx. 7.4. The KCl depolarizing solution had the same composition as the above except that the NaCl was replaced by an equimolar amount of KCl. Muscle samples were frozen at appropriate times after addition of drugs or KCl depolarizing solutions by means of a Wollenberger-type clamp precooled in isopentane at −80°C (Diamond & Brody, 1966).

Cyclic nucleotide levels in the frozen muscles were determined essentially as previously described (Diamond & Hartle, 1974). Briefly, the samples were homogenized in 5% trichloroacetic acid (TCA) and the TCA removed by ether extraction. Cyclic nucleotide levels in the aqueous extracts were then determined by radioimmunoassay techniques as originally described by Steiner, Parker & Kipnis (1972). Aliquots of the samples were acetylated as suggested by Harper & Brooker (1975) in order to increase the sensitivity of the cyclic GMP assay. Results are reported as picomoles cyclic nucleotide per g wet wt. of tissue.

Phosphorylase activity in the muscles was determined as described elsewhere (Diamond & Brody, 1965). Activity was measured in the presence of 0.1 mM adenosine monophosphate (AMP) (which is a measure of total phosphorylase activity) and in the absence of added AMP (which is a measure of the active or *a* form of phosphorylase). Results are expressed as the percentage of the total enzyme present in the active form (% *a*).

Solutions of verapamil (Knoll Pharmaceuticals Company) and hydralazine (Sigma Chemical Company) were prepared fresh daily by dissolving them in the appropriate physiological salt solutions. They were added directly to the muscle baths to give the final drug concentrations listed in Results.

Data were evaluated statistically by means of Student's *t* test. The 0.05 level of probability was accepted as statistically significant.

Results

Effects of KCl and calcium on tension and cyclic GMP levels in guinea-pig taenia coli

After equilibration in normal physiological salt solution for 45 to 60 min, isolated strips of guinea-pig taenia coli were depolarized by replacing the bathing medium with one containing 124 mM KCl as described in Methods. Cyclic GMP levels and isometric tension in strips clamp-frozen at various times after KCl-depolarization are compared in Figure 1. There was a rapid, transient increase in tension immediately after exposure to the high KCl solution. Tension reached a peak at approx. 15 s, then decreased toward control and had levelled off in a sustained contracture within 10 min after KCl-depolarization. The increase in tension was accompanied by a similar, rapid rise in cyclic GMP levels which appeared to reach a peak at approx. 30 s after KCl addition. Levels of the cyclic nucleotide subsequently decreased toward control and had also levelled off within 10 min at an intermediate value approx. 275% above control. Cyclic GMP levels in the normally-polarized control groups

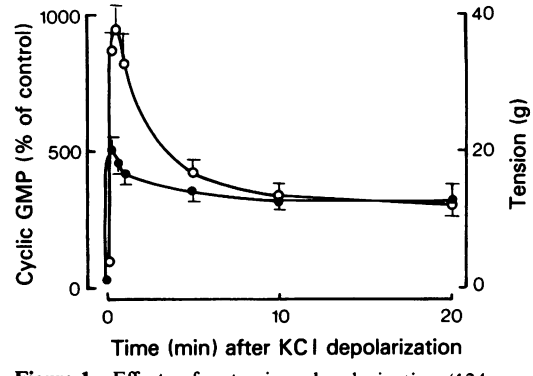

Figure 1 Effects of potassium-depolarization (124 mM KCl) on tension and cyclic GMP levels in guinea-pig taenia coli. Tension, g (●); cyclic GMP, % of control (○). Points represent means of 8 to 17 experiments. Vertical lines represent s.e. means.

used in these experiments ranged from 11 to 16 pmol/g wet wt. of tissue.

Since KCl-induced increases in cyclic GMP levels in another type of smooth muscle, the rat vas deferens, had been previously reported to be dependent on extracellular calcium (Schultz, Hardman, Schultz, Baird & Sutherland, 1973), the role of calcium in the cyclic GMP response to KCl in the taenia coli was also studied. Strips of taenia coli were equilibrated for 1 h in calcium-free physiological salt solution containing 30 μM EGTA. This treatment completely abolished spontaneous motility in the taenia and, in agreement with the results of Schultz *et al.* (1973), appeared to lower cyclic GMP levels (compare control values in Table 1 with those in Table 2). Depolarization of these muscles with 124 mM KCl had no effect on either tension or cyclic GMP levels in the absence of extracellular calcium (Table 1). Addition of 2.5 mM $CaCl_2$ to the calcium-deficient muscles caused a significant increase in tension and cyclic GMP levels within 1 min. Addition of the same concentration of $CaCl_2$ to calcium-deficient muscles in the presence of 124 mM KCl (which increases the permeability of the cell membrane to calcium) resulted in a much larger increase in tension and a marked increase in cyclic GMP concentration. These results indicate that the increases in cyclic GMP that occur during KCl-induced contractions of guinea-pig taenia voli are calcium-dependent.

Effects of calcium removal and relaxant drugs on tension and cyclic nucleotide levels in KCl-depolarized guinea-pig taenia coli

A series of experiments was designed to test the effects of verapamil, hydralazine and calcium removal on cyclic nucleotide levels and tension in KCl-depolarized guinea-pig taenia coli. A representative protocol for one of these experiments is illustrated in Figure 2.

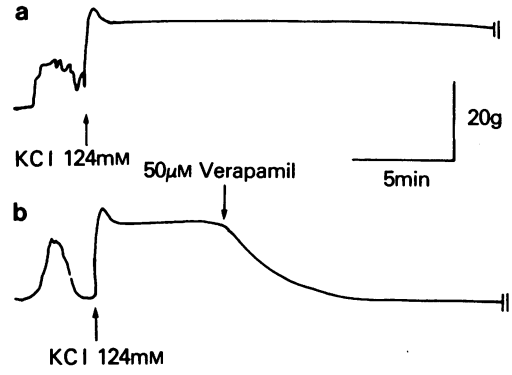

Figure 2 Illustration of experimental protocol. (a) Illustrates KCl control muscle depolarized by exposure to 124 mM KCl and clamp-frozen 20 min later at point indicated by double line; (b) shows the effect of 50 μM verapamil added to bath 6 min after KCl-depolarization of muscle strip. Muscle was clamp-frozen 14 min after addition of verapamil.

Control muscles were depolarized by exposure to 124 mM KCl and clamp-frozen 20 min later. Test muscles were also exposed to 124 mM KCl for 20 min and, in addition, were exposed to 50 μM verapamil, 2 mM hydralazine or zero calcium for the last 14 min of the experiment. Panel b of Figure 2 illustrates the effect of verapamil on isometric tension in the KCl-depolarized taenia coli. A similar response was obtained with calcium removal or hydralazine although the onset of relaxation was usually somewhat slower with hydralazine and relaxation was not always complete.

The effects of these procedures on tension and cyclic nucleotide levels in muscles frozen 20 min after KCl depolarization are shown in Table 2. Cyclic nucleotide levels in a group of relaxed, normally-polarized muscles assayed concurrently are included for comparison. After a 20 min exposure to 124 mM KCl,

Table 1 Effects of potassium chloride and calcium chloride on tension and cyclic GMP levels in guinea-pig taenia coli

Treatment	n	*Cyclic GMP (pmol/g tissue)*	*Tension (g)*
Calcium-free controls	5	2.4 ± 0.4	0.0
Calcium-free plus KCl (124 mM, 2 min)	6	2.8 ± 0.4	0.0
Calcium-free plus $CaCl_2$ (2.5 mM, 1 min)	5	11.8 ± 3.0*	2.1 ± 0.6*
Calcium-free plus KCl (124 mM, 2 min) plus $CaCl_2$ (2.5 mM, last 1 min)	6	51.8 ± 11.2*	9.7 ± 1.9*

All muscles were equilibrated for 1 h in calcium-free physiological salt solution containing 30 μM FGTA. One group of muscles was frozen without further treatment and used as calcium-free controls. Other groups were exposed to KCl and/or $CaCl_2$ as indicated. Results represent means ± s.e. of the number of experiments shown (*n*).
* Significantly different from calcium-free controls ($P < 0.02$).

cyclic GMP levels were increased by approx. 250% above control values. Although increases in cyclic AMP were seen at earlier times after KCl depolarization (Diamond & Janis, unpublished observations), the levels of this cyclic nucleotide were not significantly different from controls after 20 min of exposure to the high KCl solution. When the usual KCl-depolarizing solution was replaced by one containing no calcium, the depolarized muscles immediately began to relax. After 14 min in the calcium-free depolarizing solution both tension and cyclic GMP levels had returned to control values. Similarly, addition of 50 μM verapamil to depolarized muscles completely relaxed the muscles and decreased their cyclic GMP content to control levels within 14 min. Addition of 2 mM hydralazine produced almost complete relaxation of the depolarized muscles but did not decrease their cyclic GMP concentration. Simultaneous addition of hydralazine and removal of calcium from the depolarizing solution completely relaxed the muscles and decreased cyclic GMP levels to control. Hydralazine did not increase the cyclic GMP content of normally-polarized muscles. The latter observations indicate that hydralazine does not have any direct effect on cyclic GMP levels in the taenia coli. No significant changes in cyclic AMP levels were observed in any of these experiments (Table 2).

In the previous experiments calcium removal or verapamil addition completely relaxed the depolarized muscles within 14 min whereas hydralazine caused only a 94% relaxation of the muscles (Table 2). To determine whether it was necessary to produce complete relaxation in order to decrease cyclic GMP levels, the following experiment was performed: muscles were frozen at earlier times (i.e. 4 min) after calcium removal or verapamil administration to depolarized muscles, at which time the muscles were only partially relaxed (i.e. 77 and 84% respectively). In both cases a significant decrease in cyclic GMP levels was seen relative to the levels in the corresponding KCl controls (Table 3). Thus, it was not necessary to produce complete relaxation of the muscles in order to demonstrate a fall in cyclic GMP levels after verapamil administration or calcium removal. A 6 min exposure to hydralazine caused a similar degree of relaxation without lowering cyclic GMP levels (Table 3).

Effects of calcium removal and relaxant drugs on tension and phosphorylase activity in KCl-depolarized guinea-pig taenia coli

Previous studies had indicated that glycogen phosphorylase activity was increased during contractions of guinea-pig isolated taenia coli and that the magnitude of the increase in phosphorylase activity was directly related to the concentration of calcium in the bathing medium (Diamond, 1973). Thus, increases in both phosphorylase activity and cyclic GMP levels appear to be calcium-dependent phenomena in guinea-pig taenia coli. Therefore, the experiments described in Table 3 were repeated but this time phosphorylase activity, rather than cyclic GMP concentration, was measured in the frozen muscle samples.

Table 2 Effects of hydralazine, verapamil and calcium removal on tension and cyclic nucleotide levels in potassium-depolarized guinea-pig taenia coli

Treatment	n	*Cyclic AMP (pmol/g tissue)*	*Cyclic GMP (pmol/g tissue)*	*Relaxation (%)*
Normally polarized control	8	447 ± 39	10.1 ± 1.9*	—
KCl control (124 mM, 20 min)	9	484 ± 32	35.0 ± 6.1	—
KCl (124 mM, 20 min) minus Ca^{2+} (0 Ca^{2+}, last 14 min)	8	417 ± 38	12.8 ± 1.5*	100*
KCl (124 mM, 20 min) plus verapamil (50 μM, last 14 min)	8	420 ± 38	12.8 ± 1.9*	100*
KCl (124 mM, 20 min) plus hydralazine (2 mM, last 14 min)	8	441 ± 77	37.8 ± 5.6	94 ± 2*
KCl (124 mM, 20 min) plus hydralazine, minus calcium (2 mM hydralazine and 0 Ca^{2+}, last 14 min)	7	430 ± 37	12.4 ± 1.0*	100*
Normally polarized plus hydralazine (2 mM, 14 min)	8	425 ± 31	12.3 ± 1.5*	—

Values for relaxation refer to the % relaxation of KCl-induced contractures produced by the respective treatments. Spontaneous contractile activity was abolished in normally-polarized muscles exposed to hydralazine.
* Significantly different from KCl controls ($P < 0.01$).

The results were essentially the same as those obtained in the cyclic GMP experiments. After a 20 min exposure to potassium-depolarizing solutions, phosphorylase *a* activity in the taenia was increased by several fold over that in the normally-polarized controls (Table 4). Removal of calcium from the depolarizing solutions, or addition of 50 μM verapamil, completely relaxed the depolarized muscles and decreased the phosphorylase activity to control levels. Addition of 2 mM hydralazine, on the other hand, markedly relaxed the muscles without lowering phosphorylase activity. The concomitant administration of hydralazine and removal of calcium from the depolarizing solution relaxed the taenia and decreased phosphorylase *a* activity to control levels.

Discussion

As mentioned above, it has been suggested by Schultz and coworkers (1977) that increases in cyclic GMP levels may be responsible for the smooth muscle relaxant effects of drugs such as hydralazine and D-600 (a methoxy derivative of verapamil). We have already provided some evidence against this hypothesis in rat vas deferens where hydralazine and verapamil were found to relax KCl-contracted muscles without further increasing cyclic GMP levels, which were already elevated by the KCl (Diamond & Janis, 1978). In the present study, similar results were obtained in another type of smooth muscle, the guinea-pig taenia coli. As was the case with vas deferens, both hydrala-

Table 3 Effects of hydralazine, verapamil and calcium removal on tension and cyclic GMP levels in potassium-depolarized guinea-pig taenia coli

Treatment	n	*Cyclic GMP* (*pmol/g tissue*)	*Relaxation* (%)
KCl control (124 mM, 10 min)	6	44.1 ± 8.9	—
KCl (124 mM, 10 min) plus hydralazine (2 mM, last 6 min)	6	44.9 ± 5.9	70 ± 4*
KCl control (124 mM, 10 min)	7	50.5 ± 6.3	—
KCl (124 mM, 10 min) plus verapamil (50 μM, last 4 min)	6	30.5 ± 4.9*	84 ± 3*
KCl (124 mM, 10 min) minus Ca^{2+} (0 Ca^{2+}, last 4 min)	6	29.0 ± 7.0*	77 ± 4*

* Significantly different from the corresponding KCl controls ($P < 0.05$).

Table 4 Effects of hydralazine, verapamil and calcium removal on tension and phosphorylase activity in potassium-depolarized guinea-pig taenia coli

Treatment	n	*Phosphorylase* (% *a*)	*Relaxation* (%)
Normally polarized control	3	4.2 ± 1 5*	—
KCl control (124 mM, 20 min)	7	15.3 ± 2.5	—
KCl (124 mM, 20 min) minus Ca^{2+} (0 Ca^{2+}, last 14 min)	6	3.2 ± 1.1*	100*
KCl (124 mM, 20 min) plus verapamil (50 μM, last 14 min)	5	5.5 ± 1.8*	100*
KCl (124 mM, 20 min) plus hydralazine (2 mM, last 14 min)	7	14.2 ± 1.5	84 ± 3*
KCl (124 mM, 20 min) plus hydralazine, minus calcium (2 mM hydralazine and 0 Ca^{2+}, last 14 min)	6	3.9 ± 1.1*	100*

* Significantly different from KCl controls ($P < 0.01$).

zine and verapamil markedly relaxed KCl-depolarized guinea-pig taenia coli without further increasing tissue levels of cyclic GMP. Thus, at least under these conditions, increases in cyclic GMP levels do not appear to be responsible for the smooth muscle relaxant effects of these agents. Previous reports have also noted that marked increases in cyclic GMP levels can be produced in rat vas deferens by sodium nitroprusside (Diamond & Janis, 1978) and in canine femoral artery by carbachol and methacholine (Diamond & Blisard, 1976) with no demonstrable relaxation of these tissues. These observations, together with the present results, tend to argue against a general role for cyclic GMP as a mediator of smooth muscle relaxation.

In the experiments described in the present paper cyclic GMP levels were not increased when muscles were relaxed by verapamil but, on the contrary, they were decreased. This is the expected observation if one assumes that (1) the increased cyclic GMP level observed in the taenia coli during a KCl contracture is a result of the increased cytoplasmic calcium concentration which accompanies the contracture and (2) that the relaxation caused by verapamil is accompanied by a decrease in the cytoplasmic calcium concentration. Some evidence for the first assumption was provided in the experiments shown in Table 1, where removal of calcium from the bathing medium prevented the KCl-induced increase in cyclic GMP content and readdition of calcium in the presence of KCl caused a marked increase in the concentration of the cyclic nucleotide. This agrees with previous reports in other types of smooth muscle (Schultz *et al.*, 1973; Clyman, Blacksin, Sandler, Manganiello & Vaughan, 1975) where KCl-induced increases in cyclic GMP levels were also shown to be calcium-dependent. Evidence for the second assumption has been provided in experiments such as those of Kroeger, Marshall & Bianchi (1975) who found that calcium antagonists such as verapamil and D-600 could inhibit depolarization-induced calcium influx in some types of smooth muscle. This would result in a decrease in the cytoplasmic calcium concentration in the depolarized muscles and would account for the fall in cyclic GMP levels observed in the present experiments. Similarly, any other agent or condition which lowers intracellular ionized calcium in the depolarized muscles should produce both a relaxation of the muscles and a fall in cyclic GMP levels. As shown in Table 2, removal of calcium from the KCl-depolarizing solution did exactly that, providing further evidence that changes in cyclic GMP levels in the depolarized muscles are a reflection of changes in cytoplasmic calcium concentration. Relaxation of the depolarized muscles with hydralazine, on the other hand, was not accompanied by a fall in cyclic GMP. The simplest interpretation of this observation is that hydralazine relaxed the taenia coli without lowering the intracellular ionized calcium concentration. However, this result might also have been obtained if hydralazine itself increased cyclic GMP levels by a direct action on the cyclic nucleotide system. Evidence against this possibility is provided in Table 2. First, hydralazine had no demonstrable effect on cyclic GMP levels in normally-polarized taenia coli. Secondly, cyclic GMP levels did fall when hydralazine was added to the depolarized muscles in the absence of extracellular calcium. The latter result indicates that hydralazine cannot maintain elevated cyclic GMP levels when cytoplasmic calcium concentration is decreased, again suggesting that hydralazine itself does not decrease the calcium concentration. Another possible explanation for the observed results is that there is a marked difference between the sensitivity of the contractile elements and the sensitivity of the guanylate cyclase system to calcium. If this were true, hydralazine might possibly have lowered calcium below the threshold for activation of the contractile elements but not below the threshold for activation of guanylate cyclase. Verapamil administration and calcium removal, on the other hand, might have lowered the calcium to a greater extent i.e. below the threshold for either system. This would be consistent with the observation that hydralazine did not produce complete relaxation under these conditions (although it did relax the muscles by about 94%) whereas verapamil or calcium removal completely relaxed the muscles. However, as indicated in Table 3, partial relaxation of the depolarized muscles at earlier times after verapamil administration or calcium removal was accompanied by a partial decrease in cyclic GMP levels. Therefore, the 94% relaxation produced by hydralazine should have resulted in some fall in cyclic GMP levels if it was, in fact, due to a decrease in cytoplasmic calcium concentration. Finally, although we are not aware of any direct evidence for it, a third possible explanation for our results is that the increase in cyclic GMP levels seen during a KCl contracture may be a result of the increased calcium influx itself, and not the increased cytoplasmic calcium concentration. Verapamil and calcium-free solutions would therefore lower cyclic GMP levels by decreasing calcium influx, but hydralazine might reduce cytoplasmic calcium without decreasing cyclic GMP levels if calcium influx were unchanged. This mechanism would require that guanylate cyclase be membrane bound since it would have to respond to depolarization-induced calcium influx and not to cytoplasmic calcium levels. However, it has been demonstrated in a variety of tissues that the soluble form of guanylate cyclase, and not the particulate one, is stimulated by calcium (Mittal & Murad, 1977). Similarly, the results of the phosphorylase experiments (Table 4) also tend to argue against this hypothesis

since phosphorylase is not localized in the cell membrane and should respond to changes in cytoplasmic calcium concentration rather than to changes in calcium influx itself. As indicated above, the results of these experiments (in which a second calcium-dependent enzyme activation was monitored) were essentially the same as those obtained in the cyclic GMP experiments. In our opinion, the most likely explanation for these results is that verapamil relaxes the depolarized muscles by lowering cytoplasmic calcium concentration whereas hydralazine relaxes the muscles without lowering the calcium levels. This implies that hydralazine must exert its relaxant effects in these preparations at a site in the excitation-contraction coupling scheme beyond the regulation of cytoplasmic calcium levels, i.e. by affecting the metabolism of the cell or by an action on the contractile proteins themselves. However, at the present time we have no direct evidence to support either suggestion.

Two other recent papers have presented data bearing on some aspects of this problem but have come to conclusions different from ours. In one of these reports Sprügel, Mitznegg & Heim (1977) noted that decreases in tissue cyclic GMP levels occurred during relaxation of guinea-pig ileum by another calcium antagonist, fendiline. Our results with verapamil in the taenia coli are consistent with this observation but our interpretation of the results differs from that of Sprügel *et al.* The latter authors concluded that their data support the suggestion that cyclic GMP levels play an important role in the control of smooth muscle motility. They further suggested that an increase in the intracellular level of cyclic GMP will initiate a smooth muscle contraction whereas a decrease in cyclic GMP (as with fendiline) will result in relaxation. We have previously argued against a role for cyclic GMP as a mediator of smooth muscle contraction (Diamond, 1978) and, in our opinion, the decrease in cyclic GMP levels observed by Sprügel *et al.* during fendiline-induced relaxation of the ileum, is more likely to be a result of the relaxation (as discussed above) than a cause of it.

In another recent paper, McLean, du Souich, Barron & Briggs (1978) studied the effect of hydralazine on KCl contractures in another type of smooth muscle, the rabbit aorta. These authors found that KCl-induced increases in calcium influx (as measured by the lanthanum technique) were decreased by relaxant concentrations of hydralazine. It was suggested that hydralazine inhibited potassium-induced contractures in aortic strips by an action at the cell membrane, interfering with the entry of calcium into the cells and thereby blocking access of calcium to the contractile elements. Our results in the taenia coli are not consistent with this hypothesis but suggest, as discussed above, that hydralazine relaxes the taenia coli without affecting tissue calcium levels. It should be emphasized that results obtained in one type of smooth muscle do not necessarily apply to other types of smooth muscle. It is therefore difficult to compare these two studies since not only different tissues but different experimental approaches were used. Further experiments will be needed before it can be determined whether or not changes in cytoplasmic calcium levels play an important role in the smooth muscle relaxing action of hydralazine.

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