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SHORT COMMUNICATION

Effects of Stimulant and Relaxant Drugs on Tension and Cyclic Nucleotide Levels in Canine Femoral Artery

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SUMMARY

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Cyclic nucleotide levels and isometric tension were monitored simultaneously in isolated strips of canine femoral arteries after exposure to various drugs. Phenylephrine (5 μM) produced sustained contractions of the vascular strips but had no significant effect on tissue levels of guanosine 3',5'-cyclic monophosphate or adenosine 3',5'-cyclic monophosphate. Carbachol (100 μM), which had no effect on the tension developed by the arteries, significantly increased tissue levels of cyclic GMP but not of cyclic AMP. Papaverine (100 μM) and nitroglycerin (200 μM) both consistently relaxed phenylephrine-contracted femoral arteries and significantly increased their cyclic GMP levels. Cyclic GMP levels were increased by as much as 73% during papaverine-induced relaxation, and by 1540% during nitroglycerin-induced relaxation. Neither drug had any significant effect on cyclic AMP levels under these conditions. These results are not consistent with the earlier suggestion that contraction and relaxation of vascular smooth muscle are mediated by increases in tissue levels of cyclic GMP and cyclic AMP, respectively.

It has recently been suggested that changes in tissue levels of the cyclic nucleotides act reciprocally in the regulation of vascular smooth muscle tone, with increases in guanosine 3',5'-cyclic monophosphate promoting contraction and increases in adenosine 3',5'-cyclic monophosphate promoting relaxation of vascular smooth muscles (1-4). This suggestion is based on reports that cyclic GMP levels in vascular strips can be increased by various drugs known to be capable of contracting vascular smooth muscles (1-3) and that cyclic AMP levels can be increased by a variety of vasodilator drugs (1-3, 5-9). It has been further suggested that changes in the ratio of cyclic GMP to cyclic AMP may be more important in the control of vascular tone than changes in absolute levels of either cyclic nucleotide alone (1, 2, 4). This hypothesis has recently been extended in an attempt to explain the etiology of essential hypertension. For example, Amer *et al.* (4) reported that the cyclic GMP to cyclic AMP ratios in aortae and mesenteric arteries from spontaneously hypertensive rats were significantly higher than those in the corresponding controls. They suggested that these changes could provide the biochemical basis for the increased vascular smooth

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muscle tone and peripheral resistance observed in hypertensive rats. Similar conclusions were reached by Ramanathan and Shibata (10), who reported lower levels of cyclic AMP in vascular smooth muscles from hypertensive rats compared with their normotensive controls. These conclusions regarding the etiology of essential hypertension are based on the assumption that the cyclic nucleotides do, in fact, control smooth muscle tension as suggested by others. However, in our opinion this relationship has not been definitely established. The experiments described in the present communication were designed to compare (in the same muscles) changes in tension with changes in tissue levels of the cyclic nucleotides, after exposure of the muscles to various stimulant and relaxant drugs. The results obtained do not support the earlier suggestions that changes in the absolute levels of cyclic nucleotides, or in the ratio of cyclic GMP to cyclic AMP, play an important role in the control of vascular tone.

Segments of femoral arteries were obtained, under pentobarbital anesthesia, from male mongrel dogs weighing 10–20 kg. Helically cut strips of the arteries were prepared as described by Furchgott and Bhadrakom (11). The vascular strips so obtained were suspended at 37° in isolated organ baths containing 10 ml of a physiological salt solution with the following composition: NaCl, 118 mM; KCl, 5.7 mM; $MgSO_4$, 2.33 mM; $CaCl_2$, 1.26 mM; $NaHCO_3$, 25 mM; NaH_2PO_4, 1.17 mM; and glucose, 11 mM. The muscles were aerated by bubbling with a mixture of 95% O_2 and 5% CO_2, and isometric tension was recorded as previously described (12). A stable resting tension of 2 g was applied to the vascular strips, and they were allowed to equilibrate for at least 2 hr in the normal physiological salt solution. Samples were then clamp-frozen, either without further treatment or at various times after addition of drugs to the muscle baths. Cyclic nucleotide levels and tension at the time of freezing could then be compared in the same muscles. Although the experiments were not done on a strictly paired basis, at least one control strip (in addition to several test strips) was taken from each artery used in the study, so that control and drug-treated muscles were obtained from the same population of arteries.

Cyclic nucleotide levels in the frozen muscles were determined as previously described (13), with the addition of a succinylation step as suggested by Frandsen and Krishna (14) to increase the sensitivity of the cyclic GMP immunoassay. Levels of the cyclic nucleotides are expressed as picomoles per gram of tissue, wet weight.

The effects of various drugs on isometric tension of isolated canine femoral arteries are illustrated in Fig. 1. Vascular relaxation similar to that illustrated for nitroglycerin was also obtained with papaverine (not shown), although the onset and rate of relaxation were somewhat slower with papaverine than with nitroglycerin.

Arterial strips were clamp-frozen 5 sec and 5 min after addition of 5 μM phenylephrine to the muscle baths. This concentration of phenylephrine had been found, in separate experiments, to produce consistent, submaximal contractions of the canine femoral arteries. The muscles had just begun to contract within 5 sec after adding the phenylephrine to the baths, and developed peak tension within 3–5 min (Fig. 1). Peak tension, once attained, remained constant for at least 10 min. Cyclic GMP and cyclic AMP levels in muscles frozen 5 sec and 5 min after addition of phenylephrine are shown in Table 1. No significant changes in the levels of either cyclic nucleotide could be detected at these times, even though increases in tension were evident. If increases in cyclic GMP levels were in fact responsible for the observed contractions, then cyclic GMP levels should have been increased in the ar-

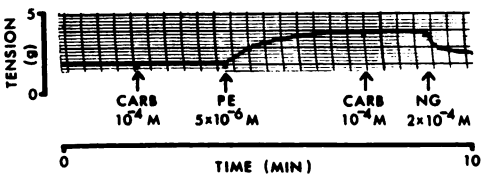

FIG. 1. *Representative tracing illustrating effects of phenylephrine (PE), carbachol (CARB), and nitroglycerin (NG) on tension of helically cut strips of canine femoral artery*

TABLE 1

Effect of stimulant and relaxant drugs on tension and cyclic nucleotide levels in canine femoral artery

Values represent means ± standard errors of the number of experiments indicated (*N*). Concentrations of drugs used were: phenylephrine, 5 μM; carbachol, 100 μM; papaverine, 100 μM; nitroglycerin, 200 μM. In the experiments in which drugs were added to phenylephrine-contracted arteries, the tension changes listed represent the effect of the second drug on phenylephrine-stimulated tension.

Treatment	*N*	Cyclic GMP	Cyclic AMP	Change in tension
		pmoles/g tissue		*g*
Control	23	8.1 ± 0.7	384 ± 37	
Phenylephrine, 5 sec	6	7.2 ± 1.2	422 ± 72	+0.10 ± 0.02
Phenylephrine, 5 min	19	7.9 ± 0.8	444 ± 54	+1.54 ± 0.33
Carbachol, 5 min	7	12.8 ± 0.8[a]	493 ± 55	0.0
Phenylephrine, 5 min; carbachol, last 1 min	9	17.6 ± 2.5[a]	451 ± 45	0.0
Phenylephrine, 5 min; papaverine, last 1 min	6	11.9 ± 1.5[a]	409 ± 46	−0.15 ± 0.04
Phenylephrine, 5 min; papaverine, last 2 min	5	13.7 ± 2.1[a]	379 ± 48	−0.57 ± 0.10
Phenylephrine, 5 min; nitroglycerin, last 1 min	10	129.8 ± 25.2[a]	433 ± 45	−0.36 ± 0.06

[a] Significantly different from controls or from phenylephrine alone ($p < 0.05$).

teries at (or slightly before) the onset of the contractions. It is apparent from the data in Table 1 that this was not the case. Earlier reports (15–17) indicated that cyclic GMP levels are increased in many nonvascular smooth muscle preparations after exposure to high concentrations of cholinergic drugs. In the present study concentrations of carbachol as high as 100 μM had no effect on the tension developed by canine femoral arteries, either at rest or during phenylephrine-induced contraction (Fig. 1). However, in agreement with the earlier reports on other types of smooth muscle (15–17), cyclic GMP levels (but not cyclic AMP levels) were significantly increased by carbachol (Table 1). For example, in the experiments with resting arterial strips, cyclic GMP levels were significantly increased by 5 min after the addition of carbachol, although no changes in tension were observed during that time. Similar results (not shown) were obtained in preliminary experiments with methacholine, a muscarinic agonist with relatively little nicotinic activity. Methacholine, in a concentration of 100 μM, had no effect on tension of phenylephrine-stimulated arteries, but increased the cyclic GMP level by approximately 2-fold within 1 min. Thus, in the case of phenylephrine, we were able to contract the muscles without increasing cyclic GMP levels, and in the case of carbachol and methacholine we were able to increase cyclic GMP levels without contracting the muscles. These results are not consistent with a general role for cyclic GMP as a mediator of vascular contraction.

The effects of two common vasodilators (papaverine and nitroglycerin) on tension and cyclic nucleotide levels in canine femoral arteries are also shown in Table 1. These drugs have been suggested by others to relax vascular smooth muscles by virtue of their ability to increase cyclic AMP levels in the muscles (5, 8). However, no significant changes in cyclic AMP levels were detected in our experiments within 1–2 min after addition of the drugs to phenylephrine-contracted muscles, even though both drugs were exerting a relaxant effect at those times. The concentrations of the drugs used in these experiments were shown in separate studies to be capable of causing 80–90% relaxation of the phenylephrine-contracted arterial strips within 5 min. With the same analytical techniques used in the present experiments, we have been able to detect changes in cyclic AMP levels in other types of smooth muscle in response to various drugs (e.g., refs. 13, 18). Thus increases in cyclic AMP levels probably do

not play a prominent role in the vascular relaxation caused by papaverine or nitroglycerin in the present experiments. Furthermore, cyclic GMP levels were significantly increased by both relaxant drugs (Table 1). The 16-fold increase in cyclic GMP levels caused by nitroglycerin is the largest increase observed in any of our experiments and tends to argue against a role for cyclic GMP as a mediator of smooth muscle contraction, since the muscles were in the process of being relaxed by nitroglycerin at the time of freezing. We have previously reported a similar lack of correlation between tension and cyclic nucleotide levels in other types of smooth muscles (13, 17, 18).

The possibility that a change in the cyclic GMP to cyclic AMP ratio is the important determinant of vascular tone, as suggested by others (1, 2, 4), is not supported by our results. According to the earlier suggestion, an increase in the cyclic GMP to cyclic AMP ratio would tend to promote contraction of vascular smooth muscles, and a decrease in the ratio would tend to promote relaxation. However, in our studies, the cyclic GMP to cyclic AMP ratios actually decreased slightly during phenylephrine-induced contraction of canine femoral arteries (from 0.021 in controls to 0.017 and 0.018 at 5 sec and 5 min after phenylephrine, respectively). Cyclic GMP to cyclic AMP ratios were increased to 0.036 during papaverine-induced relaxation, and to 0.299 during nitroglycerin-induced relaxation of the arterial strips. None of these results is consistent with the above hypothesis. Therefore, in our opinion, it is premature to ascribe the increased vascular resistance seen in spontaneously hypertensive animals to an increase in their vascular cyclic GMP to cyclic AMP ratios as suggested by Amer *et al.* (4).

Our results do not, of course, rule out the possibility that other drugs, such as the *beta* adrenergic agonists, may exert their vasodilator effects by virtue of their ability to increase tissue levels of cyclic AMP. However, this possibility has recently been questioned by Daniel and Crankshaw (19), and it appears that a causal relationship between cyclic AMP increases and vasodilation has not been definitely established even for the *beta* adrenergic drugs.

It should be noted that the vascular preparations used in the present study contained more than one cell type, and the possibility exists that the changes observed in our experiments were a reflection of changes occurring in nonmuscle cells. It is also possible that the cyclic nucleotides may be compartmentalized within the cell, as suggested by others (20), and that changes in specific compartments, undetectable by total tissue measurements, may be important in the actions of the drugs tested in our experiments. However, the original theories regarding the role of the cyclic nucleotides in the control of vascular tone were themselves based on measurements of total tissue levels of cyclic nucleotides in vascular preparations similar to those used in our studies. Therefore, unless evidence can be obtained for the alternative explanations set forth above, our results suggest that in at least one type of vascular smooth muscle, and with at least some drugs, changes in tissue levels of the cyclic nucleotides do not play an important role in the control of vascular tone.

REFERENCES

1. Dunham, E. W., Haddox, M. K. & Goldberg, N. D. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, **71**, 815–819.
2. Kadowitz, P. J., Joiner, P. D., Hyman, A. L. & George, W. J. (1975) *J. Pharmacol. Exp. Ther.*, **192**, 677–687.
3. Clyman, R. I., Sandler, J. A., Manganiello, V. C. & Vaughan, M. (1975) *J. Clin. Invest.*, **55**, 1020–1025.
4. Amer, M. S., Gomoll, A. W., Perhach, J. L., Ferguson, H. C. & McKinney, G. R. (1974) *Proc. Natl. Acad. Sci. U. S. A.*, **71**, 4930–4934.
5. Kukovetz, W. R. & Pöch, G. (1970) *Naunyn-Schmiedebergs Arch. Pharmacol.*, **267**, 189–194.
6. Volicer, L. & Hynie, S. (1971) *Eur. J. Pharmacol.*, **15**, 214–220.
7. Triner, L., Vulliemoz, Y., Verosky, M., Habif, D. V. & Nahas, G. G. (1972) *Life Sci.*, **11**, 817–824.
8. Andersson, R. (1973) *Acta Pharmacol. Toxicol.*, **32**, 321–336.
9. Seidel, C. L., Schnarr, R. L. & Sparks, H. V.

(1975) *Am. J. Physiol.*, **229**, 265–269.
10. Ramanathan, S. & Shibata, S. (1974) *Blood Vessels*, **11**, 312–318.
11. Furchgott, R. F. & Bhadrakom, S. (1953) *J. Pharmacol. Exp. Ther.*, **108**, 129–143.
12. Diamond, J. & Brody, T. M. (1966) *J. Pharmacol. Exp. Ther.*, **152**, 202–211.
13. Diamond, J. & Hartle, D. K. (1974) *Can. J. Physiol. Pharmacol.*, **52**, 763–767.
14. Frandsen, E. K. & Krishna, G. (1975) *Fed. Proc.*, **34**, 231.
15. Lee, T. P., Kuo, J. F. & Greengard, P. (1972) *Proc. Natl. Acad. Sci. U. S. A.*, **69**, 3287–3291.
16. Schultz, G., Hardman, J. G., Schultz, K., Davis, J. W. & Sutherland, E. W. (1973) *Proc. Natl. Acad. Sci. U. S. A.*, **70**, 1721–1725.
17. Diamond, J. & Hartle, D. K. (1974) *Pharmacologist*, **16**, 273.
18. Diamond, J. & Holmes, T. G. (1975) *Can. J. Physiol. Pharmacol.*, **53**, 1099–1107.
19. Daniel, E. E. & Crankshaw, J. (1974) *Blood Vessels*, **11**, 295–311.
20. Andersson, R. (1972) *Acta Physiol. Scand.*, Suppl. 382, 1–59.