Mechanisms of the Contractile Effect Induced by Uridine 5'-Triphosphate in Canine Cerebral Arteries

Yoshiake Shirasawa, Ph.D., Richard P. White, Ph.D., and James T. Robertson, M.D.

SUMMARY This study was performed to elucidate mechanisms responsible for the contraction of isolated canine cerebral arteries induced by uridine 5'-triphosphate (UTP) and to ascertain whether UTP given intracerebrally causes cerebral arterial constriction. The latter was proven arteriographically to be the case. In vitro, UTP (10^(-6)M) and UDP were similar in potency, produced sustained contractions, and were more effective than other pyrimidine nucleotides or uridine. Unlike serotonin (5-HT), UTP was not antagonized by cinanserin and failed to cause constriction of mesenteric arteries. Adenosine similarly antagonized 5-HT and UTP. The Ca^2+ antagonist nimodipine abolished contractions caused by high K^+ but only incompletely antagonized 5-HT or UTP. On the other hand, procedures that hyperpolarize the cell membrane (low K^+ followed by K^+) abolished tonic contractions induced by UTP. Hyperpolarization prior to UTP (with or without nimodipine) did not, however, prevent the occurrence of a phasic contraction. Papaverine or lanthanum antagonized this phasic response. It was concluded that UTP selectively affects cerebral arteries, may initiate constriction by releasing membrane bound Ca^2+, depolarizes the cell membrane to open receptor operated and potential sensitive calcium channels, but does not inhibit the electrogenic Na-pump nor specifically antagonize the vasodilator adenosine.

The concept that nucleotides may regulate vascular tone is based upon numerous studies. It has been proposed that the intracellular nucleotide cyclic adenosine 3', 5'-monophosphate mediates relaxation of vascular smooth muscle and that cyclic guanosine 3', 5'-monophosphate production is associated with contractile responses. Recent observations, however, have shown that both of these compounds are associated with relaxation of blood vessels. Reports further suggest the nucleoside adenosine is a physiological mediator of relaxation for coronary arteries and for cerebral arteries of the cat, dog and human. The vasodilatation caused by adenosine, and closely related compounds, may be linked with the metabolic needs of the heart and brain as well as contribute to migraine. Although the physiological role of these compounds has not been established, there is agreement that the fundamental effect of purine nucleotides on cerebral arteries is relaxation. In contrast, there is little information concerning the action of pyrimidine nucleotides on the cerebral vasculature.

A report showing that small quantities of uridine 5'-triphosphate (UTP) were present in platelets prompted Urquilla to ascertain whether this nucleotide might affect isolated cerebral arteries of the dog and human. The results were remarkable in that UTP produced a prolonged vasoconstriction in these arteries, lasting about 7 and 20 hours, respectively. He suggested that UTP may be a useful tool for studying the mechanisms associated with prolonged vasospasm. Since UTP is evidently present in all cells and the brain is a rich source of this nucleotide (30 μM/100g), it is possible that under pathophysiological conditions UTP might contribute to the genesis of vasospasm. Moreover, a preliminary report by Urquilla and coworkers showed that UDP likewise caused vasoconstriction and this pyrimidine is ubiquitously present in brain and other tissue. In any case, it is clear that the effects of some pyrimidines on cerebral arteries are diametrically opposed to those produced by purine nucleotides.

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The present experiments were performed to further characterize the contractile responses produced by pyrimidines, to analyze the nature of the vasoconstriction caused by UTP, and to ascertain whether this nucleotide produced cerebral arterial spasm when injected intracisternally.

Methods

In Vitro Studies

Mongrel dogs of both sexes, weighing 15–25 kg, were anesthetized with sodium pentobarbital (30 mg/kg I.V.) and rapidly exsanguinated via the femoral artery. After brain removal, the basilar artery was exsanguinated. For some experiments the mesenteric artery (0.7–1.0 mm outside diameter) was isolated. A 4 mm length segment of the artery was mounted on rigid parallel prongs in a tissue bath containing 25 ml of nutrient solution. The bath chamber used was similar to that described by others. The composition of the Krebs-Ringer solution was as follows (in millimoles per liter): NaCl 120, KCl 4.5, CaCl 2.5, MgSO 4 1.0, NaHCO 3 27.0, KH 2 PO 4 1.0, Na 2 EDTA 0.01 and glucose 10.0. A Tris buffer Krebs-Ringer solution containing Tris chloride (25.0 mM) in place of NaHCO 3 was used when lanthanum was part of the experimental design. In some experiments 4.5 mM KCl was omitted to provide a K + deficient Krebs-Ringer solution or a K + deficient Tris buffer Krebs-Ringer solution in order to study potassium-induced relaxation. The pH of the bath solution was adjusted to 7.4. A gas mixture of 95% O 2 and 5% CO 2 routinely aerated the bath chamber and solution reservoir but 100% O 2 gas was used with the Tris buffer solution. Temperature of the water bath was maintained at 37 ± 0.05°C. The vessel was allowed to equilibrate for at least 1.5 h before the start of experiments. During this period the bath solution was replaced every 20 min. Isometric tension was recorded using a Grass polygraph (Model 7) and a Statham force displacement transducer (11388, Gl-1.5-300) held by a fine-positioning device to accurately adjust resting tension. The resting tension was adjusted to 2.5 g during equilibration.

Drugs used were uridine 5'-triphosphate (UTP), uridine 5'-diphosphate (UDP), uridine 5'-monophosphate (UMP), cytidine 5'-triphosphate (CTP), uridine, adenosine, 5-hydroxytryptamine creatine sulfate (5-HT), ouabain, norepinephrine hydrochloride, rubidium chloride, lanthanum chloride, papaverine hydrochloride (Sigma), cinanserin (E.R. Squibb & Sons, Inc.) and nimodipine (Miles Lab.). Nimodipine was dissolved in a mixture of ethanol, polyethylene glycol and water (15:15:70 by volume). This vehicle is inactive. Adenosine and uridine were dissolved in 0.1 N HCl and the solution was neutralized by 0.05 N NaOH. The other drugs were dissolved in distilled water. The solutions were added to the bath in 0.025–0.25 ml volumes.

The in vitro responses are expressed as mean ± S.E. of gram tension unless otherwise stated. Statistical analyses were made using the Student's t-test.

In Vivo Studies

The procedures used in these studies are fully described elsewhere. Briefly, mongrel dogs of both sexes, weighing from 17 to 24 kg, were anesthetized with pentobarbital sodium. Cerebral arteriograms were obtained from the injection of 5 ml of Hypaque (me-glumine diatrizoate) into the right vertebral artery before the intracisternal injection of UTP and at 5, 15, 30, 60, 120 and 180 minutes afterward. The caliber of the basilar artery was used to determine the magnitude of the responses. The UTP was administered as follows. The cisterna magna was tapped with an 18-gauge needle, 1.8 ml of CSF was collected and mixed with a 0.2 solution of UTP, and then this mixture was slowly (30 seconds) injected into the cisterna magna with the bevel of the needle positioned rostrally. In some experiments, nimodipine was also administered by this route. Systemic blood pressure and respiratory rate were recorded on a Grass polygraph. Further details are presented in Results.

Results

In Vitro Studies

1 Contractile Effect of Pyrimidine Nucleotides

All of the pyrimidine nucleotides contracted the basilar artery in a dose dependent manner but uridine, a nucleoside, had no such effect (fig. 1). None of these contractile pyrimidines was as potent as 5-HT (fig. 1). However, the maximum contraction (Cmax) generated by UTP, UDP and 5-HT was similar in magnitude. In contrast, the Cmax for UMP was significantly smaller (P < 0.01) than for UTP whereas a Cmax for CTP was unattainable in the highest concentration studied (10 -3 M). The average molar concentration (express as
— log) for each compound that produced a contractile response that was 50% of the Cmax for 5-HT was as follows: 5-HT 8.42 ± 0.15, UDP 6.80 ± 0.19, UTP 6.54 ± 0.15, UMP 4.96 ± 0.17, CPT 3.94 ± 0.21. Hence, the potency of UTP and UDP was similar but 80 times less than 5-HT while the potency of UMP and CTP was, respectively, about 3,000 and 30,000 times less than 5-HT.

The maximal contraction induced by a single application of the agonists in optimal concentrations (5-HT 10^-7M, UTP 10^-4M, UDP 10^-3M, UMP 10^-3M, and CTP 10^-3M) became evident within 1 min. The time course of these contractions varied greatly, as shown in figure 2. Both UTP and UDP produced contractions that were more sustained (tonic) than those of 5-HT. The contractions generated by UMP and CTP declined most and by 10 min had decreased to about 25% of initial value.

The contractile effect of UTP in the mesenteric artery was negligible. As shown in figure 3, both 5-HT and norepinephrine (NE) produced dose dependent responses in the mesenteric artery, whereas UTP 3 × 10^-5M produced only a negligible effect.

2 Effect of Nimodipine, Papaverine and Adenosine on the Tonic (Ongoing) Phase of UTP-Induced Contractions

These relaxants were applied 3–5 min after the basilar artery was contacted by UTP (10^-8M). At that time UTP-induced contractions are essentially steady (fig. 2) and tonic in character. These three drugs relaxed the artery in a dose dependent manner. As shown in figure 4, nimodipine was the most potent relaxant whereas papaverine was the most effective and produced complete relaxation. The maximum relaxation obtained with nimodipine was 73.3 ± 3.2% at 10^-9M; higher concentrations (10^-8M) had no further effect (fig. 4). Adenosine relaxed the artery contracted by UTP but this relaxation was not specific because it also relaxed similarly the maximal arterial contraction caused by 5-HT (10^-7M). That is, the average negative logarithmic molar concentration of adenosine that produced 50% of the maximal relaxation obtained with papaverine was 4.11 ± 0.24 in the UTP contracted artery and 4.27 ± 0.23 in arteries contracted by 5-HT (N = 6). In contrast, the 5-HT antagonist cinanserin preferentially relaxed arteries contracted by 5-HT. Thus, the negative logarithmic molar concentration of cinanserin that produced 50% of maximal relaxation was only

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**Figure 2.** Time course of the contractile effect produced by primidone nucleotides and 5-HT. Number of experiments are shown in parentheses and vertical bars represent standard errors of the mean. The maximal contraction produced by each compound was taken as 100%.

**Figure 3.** Dose-response curves comparing the contractile effect of norepinephrine (NE), 5-HT and UTP on isolated mesenteric arteries (N shown in parentheses). The maximal contraction induced by 5-HT was 26.3 ± 2.0 g and taken as 100%.

**Figure 4.** Compares the relaxant effect of nimodipine, papaverine, and adenosine on the tonic contractions of the basilar artery induced by UTP (1 × 10^-8M). Cumulative concentrations of the relaxants were applied 3–5 min after the contractile response had peaked and 2 min elapsed between this step-wise application at which time the effect plateaued. Papaverine caused complete relaxation and this effect was taken as 100%. N = 6.
7.41 ± 0.32 (N = 4) for 5-HT but much higher concentrations (1 × 10^{-5}M) failed to reduce the Cmax of UTP 50%.

3 The Comparison of Nimodipine Efficacy on Subsequent Contractions Induced by UTP, 5-HT and High K+

In these experiments the basilar arteries were preincubated for 3 min with a concentration of nimodipine (10^{-6}M), previously shown to produce maximal relaxation (fig. 4), prior to the addition of peak doses of UTP (10^{-4}M), 5-HT (10^{-6}M) or high K^+ (5.5 × 10^{-2}M KCl). As shown in figure 5, nimodipine completely antagonized the contractile response produced by high K^+ but only partially inhibited the contractions induced by UTP and 5-HT, being respectively, 48 and 41% of control. In combination with nimodipine, cinanserin (10^{-5}M) nearly abolished the responses generated by 5-HT, whereas those caused by UTP were unaffected (fig. 5).

4 UTP-Induced Contraction in Depolarized Arteries

High K^+ (5.5 × 10^{-2}M) was used to depolarize the basilar artery and produce a maximal contraction. As soon as this response had peaked, UTP (10^{-4}M) or 5-HT (10^{-6}M) was applied. As shown in figure 6, both UTP and 5-HT produced further contractions in the presence of high K^+. Moreover, high K^+ did not potentiate the responses of the agonists nor produce contractions that were as great as UTP or 5-HT (fig. 6).

5 Influence of Hyperpolarization on Arterial Contractions Produced by UTP

a. Activation of the Electrogenic Na-Pump by K^+ During UTP and 5-HT Induced Contractions. In order to reliably produce hyperpolarization (and relaxation) of the isolated vessel, the artery was bathed in a K^+ deficient Krebs-Ringer solution for 1 h. This reduces the efficiency of the electrogenic pump which in turn would increase intracellular sodium. This caused an increase in muscle tone which after 1 h of incubation average 1.19 g (N = 8) above the resting tension present beforehand in normal Krebs-Ringer buffer. This small rise in muscle tone is reversed by adding physiological concentrations of K^+ (4.5 × 10^{-3}M) or by adding Rb^+ (5 × 10^{-3}M) (fig. 7A). These ions stimulate the Na-pump under such experimental conditions. The maximal contractions generated by UTP (10^{-4}M) or 5-HT (10^{-6}M) in the K^+ deficient media were similar to those obtained in normal buffer, being 14.3 ± 1.3 and 13.9 ± 14 g, respectively. However, in the K^+ deficient solution the addition of K^+ reduced these contractions in a dose-dependent manner, with complete inhibition evident at 9 × 10^{-3}M (fig. 8). Oubain also produced contractions in the K^+ deficient media. These contractions were similar in magnitude to UTP. However, because ouabain completely inhibits the electrogenic Na-pump, the ouabain-induced response was not reversed by K^+, which contrasts with the results obtained with UTP (fig. 7B). This contrast, therefore,
clearly indicates that UTP (or 5-HT) is not an inhibitor of the vascular Na-pump.

The Affect of Membrane Hyperpolarization Prior to UTP. In these experiments hyperpolarization was produced by adding 9 \times 10^{-3} M K^+ to the tissue bath containing arteries incubated for 1 h in K^+ deficient Krebs-Ringers solution. This added K^+ produced essentially complete relaxation when applied after UTP in the K^+ deficient media (fig. 8). In the present experiments, 9 \times 10^{-3} M K^+ was given 2 min beforehand to ascertain whether the UTP contraction would be blocked by hyperpolarization. However, as shown in figure 9 (panel A), a phasic (transient) response was produced by optimal concentrations of UTP (10^{-6} M). This phasic contraction was only about 45% as great as the maximum response otherwise produced by UTP in the K^+ deficient bath (compare tracings 1 and 2). It was also reproducible every 20 min by washing with K^+ deficient Krebs-Ringers solution and repeating the sequence. Nimodipine (10^{-4} M) did not affect this phasic contraction (compare tracing 2 and 3, fig. 9A) nor does nimodipine prevent the tonic, sustained contraction produced by UTP in the K^+ deficient media (in the absence of prior hyperpolarization, tracing 4, fig. 9A). The sustained contraction is clearly abolished by K^+- induced hyperpolarization. The amplitude of the phasic and sustained contractions were however similar in the presence of nimodipine. Together, these findings suggested that the phasic response might be due to a momentary release of membrane bound or other intracellular Ca^{2+}. The fact that this transient response was blocked by papaverine (tracing 5, fig. 9A) and by lanthanum (compare 1 and 2, fig. 9B) supports this suggestion. Most of these observations are summarized in figure 10.

In Vivo Studies

The administration of 2 ml of CSF containing UTP (10^{-3} M or 10^{-4} M) intracisternally to 4 dogs produced vasoconstriction that was evident on the arteriogram taken five minutes afterward and persisted for at least 60 min (table 1). However, in 1 of 4 animals this vasospasm had decreased in magnitude at 60 min and at 3 h the maximum constriction noted in any animal was only about 7%. Three of these animals were given a second intrathecal injection of UTP and the vasoconstriction was similar in magnitude to the first administration (32.4 versus 33.1%). No such change was evident when saline was similarly administered. In 1 animal 5-HT (10^{-5} M) was injected after the UTP induced vasospasm had subsided. The vascular constriction observed arteriographically was similar with both of the agents, the maximum decrease being 35%. In 2 animals a 4 ml saline solution containing 10^{-5} M of nimodipine was injected into the cisterna magna 10 min after 10^{-2} M of UTP. In 1 of these animals the vasospasm present 5 min after UTP had decreased from 33 to 13% within 15 min of the nimodipine and in the other vasospasm of 38% completely lysed, being replaced by vasodilatation. In the latter animal, 2 h later, a second injection of UTP produced only a small narrowing of the basilar artery (6%) 5 min afterward that had disappeared by 15 min. Three and one-half hours after this administration of nimodipine, the intracisternal injection of 2 ml of blood produced a vasoconstriction of 19%. Equal volumes of the vehicle for nimodipine have no such inhibitory action. None of the drugs altered blood pressure nor respiratory rate significantly in these anesthetized animals.
The present study confirms and extends the findings of Uriquilla and associates showing that pyrimidine nucleotides produce contraction of cerebral arteries. This contractile effect is evidently specific for cerebral arteries since responses induced by UTP from isolated mesenteric (fig. 3) or coronary arteries are negligible. The absence of a pressor response upon the intravenous administration of UTP (100 µg/kg) also supports this conclusion (unpublished findings). In addition, a specific receptor seems responsible for the UTP-induced response because antagonists of serotonin (fig. 5), adrenergic agents and prostaglandin synthesis fail to selectively inhibit this response. The presence of energy rich phosphate bonds appear important determinants for the contraction produced by the uridine derivatives because UTP and UDP were more potent and produced more persistent effects than UMP, while uridine was without an effect (figs. 1 and 2). Whether pyrimidine derivatives have a similar profile of action is unknown but since CTP failed to produce sustained contractions and was only about one-thousands as potent as UTP from isolated cerebral arteries both in vivo and in vitro and there is little difference between responses produced by adenosine and adenylic nucleotides.

The various procedures used to analyze the effects of UTP on membrane potentials during contraction were based on the fundamental findings of others. The results obtained indicate that these procedures are effective in revealing multiple actions of drugs on the cell membrane using contraction and relaxation of the vascular smooth muscle as endpoints. Thus, one action of UTP was evidently to cause contraction, at least in part, via membrane depolarization that resembles high K+ and 5-HT. High K+ circumvents receptor mechanisms, will depolarize the membrane potential and produce contraction in cerebral arteries. It is thought that high K+ opens special potential sensitive Ca++ channels (PSC) to induce contractile responses because the Ca++ influx inhibitor nimodipine (and other Ca++ antagonists) abolish the effect of high K+ but only partially inhibits contractions produced by agonists like 5-HT. Serotonin also depolarizes cerebral arteries and produced contraction in cerebral arteries but specific blockers of 5-HT prevent these changes. Therefore, agonists like 5-HT appear to produce contraction via receptor operated Ca++ channels (ROC). Since nimodipine completely blocked the high K+ response and only partially inhibited the responses produced by 5-HT or UTP (fig. 5), it is likely that UTP also acts through ROC. It is also evident that maximal contraction produced by 5-HT or UTP was greater than with high K+ alone but no greater when given in combination with high K+ (fig. 6). This finding, together with the partial inhibition by nimodipine, suggests that the

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**Figure 9.** Tracings of results obtained in basilar arteries that were incubated in K+ deficient Krebs-Ringers (A) or K+ deficient Tris chloride Krebs Ringers (B) solution for 1 h previously. Bold arrows represents UTP (10^-4) administration. Tracing 1 of A shows control response to UTP in the K+ deficient media. Tracing 2 (A) illustrates that when 9 x 10^-4M of KCl is applied first to hyperpolarize the membrane, UTP only produces a phasic response. Tracing 3 demonstrates that this phasic response is not blocked by nimodipine. On the other hand, nimodipine inhibited only the maximum contraction caused by UTP but not the tonic characterist of the response, while KCl subsequently reversed all aspects of the UTP-induced contraction (tracing 4). Tracing 5 (A) demonstrates that papaverine blocks the usual phasic response of UTP in the hyperpolarized artery. Tracings at B (left to right) show the phasic response of UTP in the K+ deficient Tris chloride medium that was abolished by lanthanum.

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**Table** Magnitude and Duration of Cerebral Vasospasm Induced by UTP Given Intrathecially to Four Dogs

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<tr>
<th>Time of arteriogram after UTP injection (minutes)</th>
<th>Percent constriction (%)</th>
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*Percent constriction = (range). *

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* Sufficient UTP was mixed with 2 ml CSF to make 1 x 10^-2 and 1 x 10^-3 solutions prior to injection into the cisterna magna.
ROC mechanism can also normally open PSC to produce a more effective response.

We examined the effect of hyperpolarizing the basilar artery on the contractile response of UTP because hyperpolarization should close at least PSC and therefore, attenuate UTP-induced responses as effectively as calcium antagonists. The results obtained have substantiated this assumption and also revealed other actions of agonists. The procedure used herein (K+ deficiency followed by K+) causes hyperpolarization by activation of the electrogenic Na-pump in excitatory neurons16, 18, 27 and vascular smooth muscle.19, 20, 28, 29 The electrogenic Na-pump normally requires K+ to work efficiently to remove intracellular Na. Our results show that 1 h of incubation in a K+ deficient solution is sufficient to consistently produce relaxation of cerebral arteries upon the addition of K+ (figs. 7 & 9). The concentration of K+ added in these experiments that produced maximal relaxation in arteries contracted by UTP or 5-HT was 9 × 10^{-3}M. Similar concentrations of K+ have been reported to produce in arteries maximal hyperpolarization and relaxation20 as well as maximally stimulate ouabain-sensitive Na+ efflux.30 It is reasonable to assume, therefore, that the relaxant effect obtained by the addition of 9 × 10^{-3}M of K+ was due to membrane hyperpolarization caused by the activation of the Na-pump and concomitant extrusion of intracellular sodium. Moreover, Rb+ also stimulates the Na-pump16, 17 and, like added K+, caused relaxation (fig. 7). In contrast, ouabain is a complete inhibitor of the electrogenic Na-pump and produces depolarization of cerebral arteries5 as well as tonic contractions of these arteries.31 Oubain also produced tonic arterial contractions in K+ deficient Krebs-Ringers solution but, unlike UTP, the ouabain-induced response was not abolished upon the addition of K+ (fig. 7). This finding indicates that the tonic contraction caused by UTP was not due to inhibition of the Na-pump.

Responses obtained during hyperpolarization further indicate that the tonic sustained phase of the contraction induced by UTP is coupled to membrane depolarization since hyperpolarization promptly terminated the tonic response (fig. 7) and also prevented its occurrence (fig. 9). However, when imposed prior to the application of UTP, hyperpolarization did not prevent the appearance of a phasic response (fig. 9). The magnitude of this transient contraction was only about one-half as great as that obtained in K+ deficient or normal Krebs-Ringers solutions. Nimodipine also reduced the Cmax of UTP about one-half in either of these solutions (figs. 5, 9) and selectively blocks PSC.22, 25, 32 Thus, it is likely that hyperpolarization also closes PSC and possibly ROC. Therefore, the mechanism for the UTP phasic response appears independent of these channels because it occurred unaltered in the presence of both hyperpolarization and nimodipine (fig. 9A). Rather, it is likely due to a limited release of Ca^{2+} from storage sites associated with the cell membrane because the phasic contraction was essentially blocked by papaverine and, especially, by La^{3+} (fig. 9A and B). The marked relaxant property of papaverine has been attributed to an enhanced sequestration of intracellular Ca^{2+}, thereby reducing cytoplasmic Ca^{2+}.33 Lanthanum, however, binds at superficially located Ca^{2+} sites of the cell and blocks Ca^{2+} uptake at these sites.34, 35 Although experiments performed in Ca^{2+} free media containing chelating agents indicate that cerebral arteries utilize extracellular Ca^{2+} for contraction induced by agonists,36 our findings indicate rather that the first release of Ca^{2+} for contraction is derived from superficial sites of vascular smooth muscle. This initial release would not only begin contraction but may depolarize the membrane and open both ROC and PSC for the normal influx of Ca^{2+}. If so, hyperpolarization prevents the latter, resulting in a phasic contraction of low magnitude.

It has not been reported whether ROC is membrane potential dependent or not. As previously mentioned, this appears to be the case. Nimodipine alone blocks PSC and reduced the tonic contractions of UTP (or 5-HT) incompletely while the tonic contraction that remained (ROC) was abolished by hyperpolarization. Moreover, the finding that hyperpolarization alone could abolish and prevent the occurrence of the tonic contraction supports the conclusion that hyperpolariza-
tion close both PSC and ROC. Indeed, these pharmacologically distinct Ca\(^{2+}\) channels may normally act synergistically to produce contraction in that the opening of one perturbs the membrane sufficiently to open, at least partially, the other channel. This would explain why the maximal contraction generated by ROC (UTP or 5-HT) and by PSC (high K\(^+\)) separately were almost comparable in magnitude, yet could be preferentially inhibited (fig. 5). The fact that the simultaneous stimulation of ROC and PSC maximally did not produce an additive effect further supports this contention (fig. 6).

It has been proposed that the energy inherent in the Na\(^+\) gradient across the plasma membrane extrudes Ca\(^{2+}\) from the cell by means of a Na\(^+\)-Ca\(^{2+}\) exchange mechanism. However, the presence of this exchange mechanism in vascular smooth muscle has been seriously questioned and would not explain our findings obtained in K\(^+\) deficient media. If the Na-pump did contribute to Na-Ca\(^{2+}\) exchange, the inhibition of the Na-pump by K\(^+\) deficiency should have produced a substantial contraction. This was not the case, the contractions seen were small in magnitude (about 12% of Cmax for UTP). On the other hand, the relaxation following UTP caused by the addition of K\(^+\) in the K\(^+\) deficient bath was marked and complete (fig. 9). These changes effected by manipulating the concentrations of K\(^+\) are best explained by changes in the membrane potential. This is, in K\(^+\) deficiency the Na-pump is less efficient, the membrane is mildly depolarized, contraction is small, and Na\(^+\) slowly accumulates intracellularly with time. UTP (or 5-HT) increases this contraction (fig. 10) by producing further depolarization. Then, upon the addition of K\(^+\) the Na-pump is stimulated, hyperpolarization closes the Ca\(^{2+}\) channels, and the vessel relaxes. Also, the depolarization (and contraction) caused by K\(^+\) deficiency might be smaller than with ouabain because, in K\(^+\) deficiency, change in the membrane potential associated with inhibition of the electrogenic Na-pump may be compromised by an increase in passive K\(^+\) efflux. Such results have been obtained in taenia coli.

The arteriographic finding that intracerebral injections of UTP produce cerebral arterial constriction supports further the suggestion that this pyrimidine might contribute to the pathogenesis of cerebral vasospasm. Moreover, the absence of tachyphylaxis and the sustained action of UTP contrasts with 5-HT (fig. 2) and these are important features of putative spasmogens involved in this affliction. However, in contrast to thrombin the contraction induced by UTP is readily removed by washing. It is unclear why the duration of action in vivo of 2–3 h (table 1) is only about one-third as long as reported in vitro but this difference might be due to a washing action of CSF or to metabolic degradation. In a different milieu (as blood) its effect may be prolonged. This washing action of CSF coupled with the “irreversible” effect of nimodipine may explain why nimodipine was most efficacious in vivo. Since UDP was as effective as UTP in vitro, it might likewise contribute to pathological phenomenon. Indeed, many different pyrimidines may cause vasoconstriction and these are present in platelets, body tissues and abundantly present in brain. Whether these nucleotides are released under physiological or pathological conditions to cause vasoconstriction requires investigation. Also, it is unknown whether UTP affects the microcirculation and whether it acts synergistically with other putative spasmogens. Nevertheless, the results indicate that the procedures used in this study are effective in separating contractile responses into three components: a phasic component dependent on the release of membrane bound Ca\(^{2+}\) and the opening of two separate Ca\(^{2+}\) channels. They also show that UTP produces a selective, long-acting vasoconstrictor effect on cerebral arteries both in vitro and in vivo.

Acknowledgments

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