Precontraction with Elevated Concentrations of Extracellular Potassium Enables both 5-HT$_{1B}$ and 5-HT$_{2A}$ “Silent” Receptors in Rabbit Ear Artery

JAMES R. SMITH, CHEOL KIM, HEATHER KIM, and RALPH E. PURDY

Department of Pharmacology, College of Medicine, University of California, Irvine, California

Accepted for publication December 7, 1998 This paper is available online at http://www.jpet.org

ABSTRACT

The present study was conducted to determine the effect of a small (<10%) K$^+$-induced precontraction on the response to vasoconstrictors in the rabbit aorta and ear artery rings. In both tissues, 15 mM K$^+$ shifted the methoxamine concentration response curve (CRC) approximately 2.4-fold to the left. There was no change in the sensitivity of the control and amplified CRCs to the $\alpha_1$ adrenoceptor antagonist prazosin (100 nM). In the aorta, the CRC for serotonin was shifted 4.5-fold to the left in the presence of 15 mM K$^+$, and both the control and amplified CRCs were antagonized equally by the 5-HT$_{2A}$ antagonist ketanserin (10 nM). In contrast, 16 and 20 mM K$^+$ caused up to an approximately 60-fold leftward shift of the serotonin CRC in the rabbit ear artery. This effect of 16 mM K$^+$ was not altered by mechanical removal of the endothelium or by in vitro denervation using 6-hydroxydopamine. The K$^+$-amplified CRC was insensitive to 100 nM prazosin at serotonin concentrations below 3 $\mu$M, but was significantly antagonized by 10 nM ketanserin, suggesting that 5-HT$_{2A}$ receptors are involved in the K$^+$-amplified response. The 5-HT$_{1B}$-selective antagonist, GR 127935, did not affect control responses to serotonin, but significantly blocked the K$^+$-amplified response. Furthermore, the combination of ketanserin and GR 127935 produced a significantly greater blockade of the amplified response than either antagonist alone, supporting the conclusion that both 5-HT$_{2A}$ and 5-HT$_{1B}$ receptors mediate the K$^+$-amplified response to serotonin in the rabbit ear artery.

Serotonin is well known to exert a vasoconstrictor effect in many vascular beds, and does so generally by activating 5-HT$_{2A}$ receptors. For example, 5-HT$_{2A}$ receptors mediate vasoconstriction in the rabbit (Clancy and Maayani, 1985) and rat (Cohen et al., 1981) aorta, dog femoral artery (Peroutka, 1984), and many other vessels from several species (Cohen, 1988). Serotonin also activates 5-HT$_{1-like}$ receptors in selected vessels such as the canine saphenous vein (Humphrey et al., 1988), canine (Connor et al., 1989), rabbit (Parsons and Whalley, 1989), and human (Parsons et al., 1989) basilar arteries, and bovine pial arteries (Hamel et al., 1993a,b). Serotonin can also activate $\alpha_1$ adrenoceptors in selected rabbit blood vessels such as the rabbit aorta (Purdy et al., 1987), femoral artery (Grandaw and Purdy, 1996), and ear artery (Apperley et al., 1976; Black et al., 1981; Purdy et al., 1981; Xu et al., 1990).

Recently, there have been several reports in which serotonin or its analogs had little or no effect in an isolated blood vessel. However, if the vessel was precontracted, the tissue became more sensitive to stimulation by serotonergic agonists mediated by a previously inactive receptor. For example, sumatriptan had little or no efficacy in untreated rabbit mesenteric (Choppin and O’Connor, 1995), renal (Choppin and O’Connor, 1994), and iliac (Yildiz and Tuncer, 1995a) arteries, but elicited a potent concentration-dependent contraction if these vessels were precontracted with either a receptor agonist such as histamine or a slightly elevated concentration of K$^+$ in the bathing medium. The previously inactive receptor mediating the contraction to sumatriptan in these precontracted vessels was the 5-HT$_{1-like}$ subtype.

In the present study, receptors that are inactive in untreated blood vessels, but that are capable of becoming functional if the vessel is precontracted, are referred to as “silent” receptors. The mechanisms by which silent receptors become enabled by precontraction is unknown. Among several possibilities, they may be either poorly coupled at the second messenger level (Yildiz and Tuncer, 1995a) or exist in a low-efficacy state (Xu et al., 1990; Purdy et al., 1993). Precontraction must then either enhance the coupling or modulate the receptors to a high-efficacy state.

In the present study, the rabbit ear artery has been used to study the phenomenon of silent receptors. It has been demonstrated previously that the response to serotonin is mediated exclusively by $\alpha_1$ adrenoceptors in untreated ear artery

ABBREVIATIONS: CRC, concentration-response curve; ACH, acetylcholine; 6-OHDA, 6-hydroxydopamine; MDL 72222, 3-tropanyl-3,5-dichlorobenzoate.
rings (Purdy et al., 1981; Xu et al., 1990). However, if this vessel is pretreated with phenylephrine, the potency of serotonin is increased markedly and contractions are mediated by 5-HT1B receptors (Movahedi et al., 1995; Movahedi and Purdy, 1997). Alternatively, if the ear artery is pretreated with ouabain, the potency of serotonin is modestly increased and serotonin acts on 5-HT2A receptors (Xu et al., 1990; Purdy et al., 1993).

Several authors have used a slightly elevated $K^+$ concentration as the precontracting stimulus (Shimamoto et al., 1992, 1993; Choppin and O’Connor, 1994; Yildiz and Tuncer, 1995a). In all of these studies, the silent receptor that became enabled was the 5-HT1-like. Yildiz and Tuncer (1995b), citing their own and others’ work, have suggested that the phenomenon of enabling or activating previously silent serotoninergic receptors occurs exclusively with the 5-HT1-like subtype.

In the present study, the rabbit ear artery was precontracted with 15–20 mM $K^+$ to test for an increase in sensitivity to serotonin, and to identify which, if any, previously silent receptors become activated. These experiments also allowed us to explore further the proposition of Yildiz and Tuncer (1995b) that activation of silent receptors is related exclusively to the 5-HT1-like subtype.

**Materials and Methods**

Male New Zealand White rabbits (2–3 kg) were euthanized by exposure to 100% CO$_2$ to produce deep anesthesia (Glen and Scott, 1973), then rapidly decapitated. Thoracic aorta and ear arteries were isolated, cleaned, and cut into 3-mm rings. These rings were mounted for the measurement of isometric contraction (Bevan and Osher, 1972) in tissue baths containing 30 ml of 95% O$_2$/5% CO$_2$-gassed Krebs’ bicarbonate solution at 37°C. The composition of the Krebs’ solution in millimoles per liter was: NaCl, 119.2; KCl, 4.9; CaCl$_2$, 1.3; MgSO$_4$, 1.2; NaHCO$_3$, 25; glucose, 11.1; ascorbic acid, 0.114; and tetrasodium ethylenediamine tetracacetate, 0.03. The aorta was placed under 2g, and the ear artery under 1.5g resting force for 30 min. In preliminary experiments, these values were found to provide optimal active force development, i.e., to produce the largest repeatable contractions to a depolarizing concentration of $K^+$ (68 mM) prepared by equimolar replacement of Na$^+$ in the Krebs’ solution. Tissues were then exposed to 68 mM $K^+$-containing Krebs’ solution and allowed to contract to steady-state responses, after which the baths were drained and refilled twice with fresh Krebs’ solution. Thirty minutes later, the tissues were exposed once more to 68 mM $K^+$. In preliminary experiments, it was found that the second and subsequent exposures to 68 mM $K^+$ produced equivalent contractions in each tissue. Thus, the magnitude of the contraction to this second exposure to 68 mM $K^+$ was taken as 100% and all subsequent contractions were expressed in terms of this standard in each tissue. The resting force was readjusted as needed until the addition of agonists. Ketanserin, prazosin, 3-tropanyl-3,5-dichloro-benzoxlate (MDL 72222), and GR 127935 were added 30 min before the addition of agonists. In our hands, these exposure times were sufficient to allow the drugs to reach equilibrium at their respective concentrations. These values are expressed as the negative logarithm of the $EC_{50}$

In some experiments, a stainless steel wire was inserted into the lumen of the tissue ring and the wire was gently rolled so that the wire scraped the luminal surface to remove the endothelial layer. Successful removal of the endothelial layer was assessed by contracting the tissues with 10 $\mu$M phenylephrine, and then exposing them to 10 $\mu$M acetylcholine (ACh). Tissues that relaxed in the presence of ACh were excluded from data analysis.

In other experiments, 6-hydroxydopamine (6-OHDA) was used to denervate the artery rings according to the method of Aprigliano and Hermansmeyer (1976) as modified by Purdy et al. (1981). Briefly, the O$_2$-CO$_2$ gassers were turned off and the Krebs’ solution drained from the baths containing the ear artery rings and replaced with modified Krebs’ solution (NaHCO$_3$ omitted) adjusted to pH 4.9 and containing glucose (reduced form, 40 mg/liter) and 6-OHDA (400 mg/liter). Tissues remained in contact with 6-OHDA for 10 min, after which they were washed four times at 5-min intervals with fresh Krebs’ solution, and the O$_2$-CO$_2$ gassers were turned on. Characteristically, all tissues contracted maximally within 2 min of removal of the 6-OHDA and remained contracted up to 90 min, after which they slowly relaxed to baseline. The tissues were then washed with fresh Krebs’ solution intermittently during this time and, after returning to baseline, the resting force was re-adjusted to 1.5 g. All tissues were then exposed to 10 $\mu$M tyramine. This agent causes contractions in normal, but not denervated rabbit ear arteries (Purdy et al. 1981). In the present study, failure of 6-OHDA-treated artery rings to respond to tyramine was taken as evidence of successful denervation. The present method of denervation was shown to eliminate both neuronal uptake and electrically-stimulated release of norepinephrine in the ear artery (Purdy et al. 1981; Xu et al. 1990).

Isometric contractions were measured using Grass FT03C strain gauges (Grass Instruments, Quincy, MA) connected to Maclab data recording systems (Maclab Co., Castle Hill, Australia). All stock solutions were prepared fresh each week and were diluted daily for addition to the tissue bath in volumes of 100 $\mu$l or less. The following drugs were used in this study: serotonin creatinine sulfate, methoxamine, phenylephrine HCl, tyramine HCl, and ACH (Sigma Chemical Co., St. Louis, MO); ketanserin (a gift from Janssen Pharmaceutical Inc., Piscataway, NJ); prazosin HCl (a gift from Pfizer Inc., New York, NY); MDL 72222 and 6-OHDA (Research Biochemicals International Inc., Natick, MA); and GR 127935 (a gift from the Glaxo Pharmaceutical Co., Stevenage, UK).

Each CRC was based on at least seven artery rings from three rabbits. Statistical analysis was based on the number of animals used and CRCs were compared by repeated measures, two-way analysis of variance using SuperANOVA statistical software (Abacus Concepts Inc., Berkeley, CA). Values were considered significantly different when $p < .05$ using Scheffe’s or Student-Newman-Keuls’ posthoc tests. Shifts of CRCs along the $x$-axis were measured at the 90% level of the contraction to 68 mM $K^+$ unless otherwise stated. This level was chosen because it fell on the linear portion of each CRC in most cases, and because it was the level at which the treatment effects were the largest. When exposure to elevated concentrations of $K^+$ caused a contraction, this contraction was subtracted before plotting the contractile responses to either methoxamine or serotonin. Apparent antagonist dissociation constants ($K_B$) were determined according to the following equation: $K_B = [B]/(concentration\, ratio\,-1)$, where $[B]$ equals the antagonist concentration and the concentration ratio equals the agonist $EC_{50}$ in the presence of antagonist divided by the agonist $EC_{50}$ in the absence of antagonist. These values are expressed as the negative logarithm of the $K_B$ ($-\log K_B = pK_B$).

**Results**

The effect of slightly elevated $K^+$ concentration on the rabbit aorta contractile response to methoxamine was assessed and the results are shown in Fig. 1. Contractile CRCs

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to methoxamine were shifted 2.4-fold to the left in the presence of 15 mM K$^+$ (Fig. 1A). When control and K$^+$-amplified responses to methoxamine were obtained in the presence of prazosin (100 nM), both CRCs were shifted to the right equally, approximately 25-fold ($pK_B = 9.4$). Similar results were observed for the ear artery response to methoxamine. Namely, 16 mM K$^+$ shifted the methoxamine CRC slightly to the left and prazosin produced equivalent blockade in control and 16 mM K$^+$-treated vessels (Fig. 1B; $pK_B = 9.6$).

The effect of slightly elevated K$^+$ concentration on the contraction of rabbit aorta to serotonin was assessed and the results are shown in Fig. 2. 15 mM K$^+$ shifted the serotonin CRC to the left approximately 4.5-fold. When the 5-HT$_2$ receptor antagonist, ketanserin (10 nM), was used, this agent caused an equivalent rightward shift of the CRCs in control compared to 15 mM K$^+$-treated vessels ($pK_B = 8.6$).

In the rabbit ear artery, CRCs for serotonin were obtained in the absence and presence of 12, 16, and 20 mM K$^+$ (Fig. 3). 12 mM K$^+$ caused a 10.7-fold leftward shift of the serotonin CRC, whereas 16 and 20 mM K$^+$ caused significantly greater leftward shifts (58.2- and 77.6-fold, respectively). 20 mM K$^+$ did not cause a significantly greater shift of the serotonin CRC compared to 16 mM K$^+$; however, 20 mM K$^+$ produced a significantly greater precontraction compared to 16 mM K$^+$ (27 ± 8% versus 7.5 ± 1% of maximum, respectively; data not shown). This resulted in a decrease in the maximum obtainable response to serotonin when 20 mM K$^+$ was used. Therefore, all subsequent experiments were carried out in the presence of 16 mM K$^+$. The amplified responses tended to converge with the control responses at the higher serotonin concentrations. The control and amplified responses to serotonin were not significantly altered by either the removal of the endothelium or denervation of the tissue by exposure to 6-OHDA (data not shown).

As described in the introduction, precontraction of the rabbit ear artery with either phenylephrine or ouabain increases vessel sensitivity to serotonin, and serotonin acts on 5-HT$_{1B}$ (Movahedi et al., 1995) or 5-HT$_{2A}$ (Xu et al., 1990; Purdy et al., 1993) receptors, respectively. Thus, experiments were carried out to determine the receptor(s) mediating the response to serotonin in ear artery rings precontracted with 16 mM K$^+$. The effect of prazosin (100 nM) on the contractile response of ear artery to serotonin was assessed and the

![Fig. 1. The effect of 15 mM K$^+$ on CRCs for methoxamine in the absence and presence of 100 nM prazosin (-7 PRAZ). A, rabbit aorta; B, rabbit ear artery. N = 4/12; 68 mM K$^+$ contraction = 6.24 ± 0.20 g (A); 4.93 ± 0.16 g (B).](image)

![Fig. 2. Rabbit aorta CRCs for serotonin in the absence and presence of 15 mM K$^+$ and in the absence and presence of 10 nM ketanserin (-8 KET). N = 4/12; 68 mM K$^+$ contraction = 5.30 ± 0.16 g.](image)
results are shown in Fig. 4. In 16 mM K+-precontracted ear artery rings, prazosin had little or no effect on the serotonin CRC at concentrations below 3 μM serotonin, but caused a large rightward shift at higher serotonin concentrations (pK_B = 8.4).

The effects of the 5-HT_{2A} and 5-HT_{1B} antagonists, ketanserin (10 nM; Fig. 5) and GR 127935 (10 nM; Fig. 6), respectively, were also assessed. Both agents shifted the serotonin CRC significantly to the right in 16 mM K+-precontracted ear artery rings. The CRCs in the presence of each of these antagonists were shifted toward, but did not reach the serotonin CRCs obtained in untreated ear artery rings, at least at low serotonin concentrations. However, when ketanserin and GR 127935 were combined, the serotonin CRC in K+-precontracted ear artery rings were shifted further to the right and became superimposible with the serotonin CRC in untreated ear artery rings (Fig. 7). Qualitatively similar results were obtained in the presence of prazosin. Measured at the 30% level of contraction, the K+-amplified response to serotonin in the presence of prazosin (100 nM) was shifted significantly to the right 14-fold (p ≤ .05) in the presence of prazosin (100 nM) plus ketanserin (10 nM), and 8-fold (p ≤ .05) in the
The selectivity of GR 127935 was tested in the present study. GR 127935 had no effect on the serotonin CRC in untreated ear artery rings (Fig. 6), demonstrating a lack of effect on $\alpha$ adrenoceptors. GR 127935, up to 100 nM, also had no significant effect on the serotonin CRC in the aorta (Fig. 9), a tissue in which serotonin acts on 5-HT$_{2A}$ receptors (Feniuk et al., 1985; Purdy et al., 1987).

The effect of the 5-HT$_3$ receptor antagonist, MDL 72222, on the contractile response to serotonin was assessed in the rabbit ear artery. MDL 72222 had no effect on the serotonin CRC in either K$^+$-precontracted or untreated ear artery rings (data not shown).

Discussion

The overall goal of the present study was to identify the receptors mediating the contractile response of 16 mM K$^+$-precontracted aorta and ear artery rings to serotonin. The identity of these receptors was determined through the use of several selective receptor antagonists. Prazosin (100 nM) was used because of its known ability to cause a marked blockade of the $\alpha_1$ adrenoceptor without affecting serotonergic receptors (Purdy et al., 1987). Ketanserin (10 nM) was used because at this concentration, ketanserin selectively antagonizes 5-HT$_{2A}$ receptors, yet has no effect on $\alpha$ adrenoceptors (Purdy et al., 1987) and 5-HT$_{1A}$-like receptors (Bradley et al., 1986). 10 nM GR 127935 is selective for the 5-HT$_{1B}$ receptor and has little or no affinity for 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{3}$, and 5-HT$_{4}$ receptors (Skingle et al., 1996).

Many recent studies have presented evidence for a 5-HT$_{1A}$-like receptor in several different vascular beds (Humphrey et al., 1988; Choppin and O’Connor, 1995; Movahedi et al., 1995; Yildiz and Tuncer, 1995a,b). Moreover, a consensus is building in the literature that the vascular 5-HT$_{1A}$-like receptor is the 5-HT$_{1B}$ receptor subtype (Yildiz et al., 1998). For example, Hamel et al. (1993a) presented pharmacological data for the presence of 5-HT$_{1B}$ receptors in bovine cerebral arteries. This group later identified mRNA for the 5-HT$_{1B}$ subtype in both human and bovine cerebral arteries. Similarly, the 5-HT$_{1B}$ receptor was identified in canine coronary artery and canine saphenous vein (Cushing et al., 1994). Finally, the selective 5-HT$_{1B}$ receptor antagonist, GR 127935, significantly antagonized the canine basilar artery response to sumatriptan in a concentration range consistent with 5-HT$_{1B}$ receptor blockade (Skingle et al., 1996). Based

Fig. 7. The effect of the combination of both 10 nM GR 127935 and 10 nM ketanserin (K'/GR/KET) on K$^+$-precontracted serotonin CRCs in the rabbit ear artery. N = 4/8–12; 68 mM K$^+$ contraction = 5.04 ± 0.14g.

Fig. 8. The effect of 10 nM ketanserin (PRAZ/K'/KET) and 10 nM GR 127935 (PRAZ/K'/GR) on the K$^+$-precontracted serotonin CRC in the presence of 10 nM prazosin (PRAZ/K') in the rabbit ear artery. N = 4/8; 68 mM K$^+$ contraction = 3.52 ± 0.19g.

Fig. 9. Rabbit aorta CRCs to serotonin in the absence and presence of 1, 10, and 100 nM GR 127935 (GR). N = 4/12; 68 mM K$^+$ contraction = 4.27 ± 0.29g.
on these studies, as well as the results of the present study (Figs. 7 and 9), the designation “5-HT$_{1B}$” is used in the present paper to refer to the receptor mediating the vascular contractions that are significantly antagonized by 10 nM GR 127935.

In the present experiments, 15 or 16 mM K$^+$ was used to elicit a threshold contraction of blood vessel rings. This elevation of external K$^+$ decreases the concentration gradient for K$^+$ across the cell membrane, and this was observed to cause a modest depolarization in the muscle cells of isolated rabbit ear artery (J.R.S., unpublished results). This observation is consistent with that reported for 17 mM K$^+$ in rabbit ear artery (Droogmans et al., 1977). In turn, this depolarization is thought to cause contraction by opening voltage-dependent calcium channels (Bolton, 1979).

Modest depolarization (5–15 mV) of vascular smooth muscle cells is known to cause a small, nonspecific increase in the sensitivity of blood vessels to agonists (Fleming, 1980). For example, ouabain caused a 5- to 6-mV depolarization and increased the sensitivity of rabbit saphenous artery to noradrenaline by 1.8-fold (Abel et al., 1981). In the present study, slightly elevated external K$^+$ concentrations increased the sensitivity of both aorta and ear artery (Fig. 1) to methoxamine by 2- to 3-fold. Similarly, the sensitivity of aorta to serotonin was increased by approximately 4.5-fold. In all cases, the leftward shift was parallel and the magnitude of blockade by receptor antagonist was not changed in the presence of 15 or 16 mM K$^+$ compared to control. We propose that this sensitizing effect of elevated external K$^+$ simply reflected the nonspecific sensitization associated with partial depolarization (Fleming, 1980). Methoxamine acts as a full agonist in the rabbit aorta. In the rabbit aorta, the contractile response to serotonin is mediated through 5-HT$_{2A}$ receptors, but serotonin does not act as a full agonist (note the difference between the maximal responses to methoxamine and serotonin in control tissues). The increase in the contractile response to serotonin in the presence of elevated K$^+$ reflects an increase in the efficacy of serotonin acting at 5-HT$_{2A}$ receptors.

The form of the 16 mM K$^+$-induced sensitization of ear artery rings to serotonin differed markedly from that in aorta, or from the sensitization of both aorta and ear artery to methoxamine. The serotonin CRC in untreated ear artery rings was steep, moving from threshold to maximal contraction in approximately two orders of magnitude change in serotonin concentration. Based on Mass Law considerations, this is consistent with an action of serotonin at a single population of receptors. In contrast, the serotonin CRC in 16 mM K$^+$-precontracted ear artery rings covered nearly four orders of magnitude change in serotonin concentration. For example, compare the serotonin CRCs in the presence and absence of 16 mM K$^+$ in Fig. 5. The elongated curve in the presence of 16 mM K$^+$ suggests an action of serotonin at two or more receptors. Therefore, experiments were conducted to explore this possibility.

Prazosin (100 nM) did not block the contractile response of K$^+$-precontracted ear artery rings to serotonin below 1 μM, indicating that this phase of the serotonin CRC was not mediated by α$_1$ adrenoceptors. In contrast, this phase of the serotonin CRC was blocked by either ketanserin or GR 127935. In addition, the combination of both ketanserin and GR 127935 produced a significantly greater blockade of the response to serotonin than either antagonist alone. Similar blocking effects of either ketanserin or GR 127935 alone were obtained in K$^+$-precontracted ear artery rings in which α$_1$ adrenoceptors were blocked with prazosin. Together, these results demonstrate that the ear artery contractions to serotonin in the presence of slightly elevated potassium are mediated by both 5-HT$_{1B}$ and 5-HT$_{2A}$ receptors at serotonin concentrations below 1 μM, but by α$_1$ adrenoceptors at higher serotonin concentrations.

Yildiz and Tuncer (1995b) proposed that precontraction with either slightly elevated K$^+$ or receptor agonists enables or unmask only 5-HT$_{1-like}$ receptors. However, it is possible that these authors have studied blood vessels possessing 5-HT$_{1-like}$, but not other serotonergic receptor subtypes, in an uncoupled or silent state. The present authors have shown that the rabbit ear possesses both silent 5-HT$_{1B}$ (Movahedi et al., 1995; Movahedi and Purdy, 1997) and silent 5-HT$_{2A}$ (Xu et al., 1990; Purdy et al., 1993) receptors. Thus, the rabbit ear artery appears to be an appropriate model in which to further explore the proposition by Yildiz and Tuncer (1995b). The results of the present study clearly indicate that K$^+$ precontraction of the ear artery enables both the 5-HT$_{2A}$ and 5-HT$_{1B}$ receptors (Figs. 6 and 7).

The mechanism(s) by which precontraction with elevated K$^+$ enables previously silent receptors is/are unknown. The depolarization associated with elevated K$^+$ in the bathing medium could play a role. However, the present authors (Xu et al., 1990; Purdy et al., 1993) obtained evidence arguing against such a mechanism. Inhibition of Na$^+$ K$^+$-ATPase with ouabain was shown to depolarize the smooth muscle cells of the rabbit ear artery (Reiner, 1978) and to activate previously silent 5-HT$_{2A}$ receptors (Xu et al., 1990; Purdy et al., 1993). However, inhibition of Na$^+$ K$^+$-ATPase with dihydro-ouabain (Purdy et al., 1993) or by using 0 mM K$^+$ in the bathing medium (Xu et al., 1990) had no effect on the silent 5-HT$_{2A}$ receptors of this blood vessel. Zero mM K$^+$ causes a 10 to 15 mV depolarization of ear artery cells (Hendrickx and Casteels, 1974).

In our earlier studies (Xu et al., 1990; Purdy et al., 1993), we adopted the model of Kaumann and Frenken (1985) to explain the effect of ouabain. We proposed that the 5-HT$_{2A}$ receptor, which can exist in either a high- or low-efficacy state, resides in the low-efficacy state in the ear artery. Ouabain was proposed to enable the 5-HT$_{2}$ receptor by allosterically modulating it to the high-efficacy state. The role of such an allosteric mechanism in the present study is unknown, but cannot be ruled out.

It is also possible that the 5-HT$_{1B}$ and 5-HT$_{2A}$ receptors in the rabbit ear artery are silent because they are poorly coupled to second messenger pathways that mediate vasoconstriction. Precontraction with elevated K$^+$ may enhance the activity of critical second messenger steps, thereby improving coupling. This could arise from K$^+$-induced influx of extracellular calcium.

In conclusion, the present results demonstrate that, in rabbit ear artery, serotonin acts on both 5-HT$_{1B}$ and 5-HT$_{2A}$ receptors in the presence of 16 mM K$^+$. It is likely that the sensitivity of ear artery to serotonin is increased by 16 mM K$^+$ because the agonist has a higher affinity for 5-HT$_{1B}$ and 5-HT$_{2A}$ receptors than does for the α$_1$ adrenoceptors that mediate the response to serotonin in untreated vessels.
Acknowledgments

The authors would like to thank Natalie Ludwick for her generous assistance in the preparation of this manuscript.

References


Send reprint requests to: Dr. Ralph E. Purdy, Ph.D., Department of Pharmacology, College of Medicine, University of California, Irvine, Irvine, CA 92697-4625. E-mail: repurdy@uci.edu