Endothelium-Independent Relaxant Effect of 5-Hydroxytryptamine (5-HT) on the Isolated Rabbit Facial Vein

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Abstract

The facial vein in several species has been shown to have unusual properties, including exhibition of spontaneous myogenic tone and relaxation to norepinephrine (NE). The present study was undertaken to characterize the relaxant effect of 5-hydroxytryptamine (5-HT) on the rabbit facial vein. An isolated ring preparation of the rabbit facial vein exhibited intrinsic tone when it was stretched and the spontaneous contraction continued for hours. 5-HT concentration-dependently relaxed facial veins exhibiting spontaneous contraction. The relaxation was not inhibited by rubbing the endothelium or by NG-nitro-L-arginine (10⁻⁴ M), a nitric oxide (NO) synthase inhibitor. The 5-HT-induced relaxation was also unaffected by pretreatment with indomethacin (10⁻⁴ M), a cyclooxygenase inhibitor, and propranolol (10⁻⁴ M), a both β-adrenoceptor and 5-HT₁β-receptor antagonist. In contrast, 5-HT-induced relaxation of the facial vein was concentration-dependently antagonized by methysergide (10⁻⁷ M and 10⁻⁶ M), a non-selective 5-HT₁- and 5-HT₂-receptor antagonist, but not by NaN-190 (10⁻⁶ M) and SDZ-205,557 (10⁻⁶ M), antagonists for 5-HT₁A- and 5-HT₄-receptors, respectively. A higher (10⁻⁶ M), but not lower (3×10⁻⁷ M) concentration of ketanserin, a 5-HT₂-receptor antagonist, slightly inhibited the 5-HT-induced relaxation. These results indicate that 5-HT-induced relaxation is not due to indirect mechanisms mediated by NE released from the sympathetic nerve terminals, or by endogenous prostanoid and endothelium-derived relaxing factor (EDRF=NO) released from the vascular tissues, but due to a direct effect on the 5-HT receptors located on vascular smooth muscle cells. However, the subtype of 5-HT receptor that produces relaxation of the rabbit facial vein remains to be clarified.

Key Words: endothelium-independent relaxation, 5-hydroxytryptamine (5-HT) receptors, 5-hydroxytryptamine antagonists, methysergide, facial vein (rabbit).
Introduction

The facial vein of several animal species, including rabbits (Pegram et al., 1976), humans (Mellander et al., 1982), dogs (Tsuru and Negita, 1989) and monkeys (Chiba and Tsukada, 1990), possesses a number of unusual features. In these species the restricted buccal portion of the facial vein both exhibits myogenic tone dependent on temperature and relaxes in response to transmural nerve stimulation (TNS) and exogenous norepinephrine (NE), which is mediated via β-adrenoceptors (Pegram et al., 1976; Mellander et al., 1982; Tsuru and Negita, 1989). Thus, the facial vein is believed to have a role in cranial thermo-regulation in the rabbit (Winquist and Bevan, 1980) and the emotional blushing reaction in man (Mellander et al., 1982).

The vasoconstrictor substance serotonin released from platelets in clotting blood is now postulated to have diverse physiological roles as a neurotransmitter in the central nervous system, as a regulator of smooth muscle function in the cardiovascular and gastrointestinal systems, and as a regulator of platelet function. Thus, serotonin (5-hydroxytryptamine, 5-HT) might be important in the pathogenesis of certain vasospastic diseases and in the manifestations of carcinoid syndrome in the cardiovascular system (Sanders-Bush and Mayer, 1996). Acting via 5-HT receptors, 5-HT can directly stimulate or relax smooth muscle, influence the release of NE from adrenergic nerves, and stimulate endothelial cells to release endothelium-derived relaxing factor (EDRF) and prostaglandins (see Hollenberg, 1988). 5-HT can cause vasodilatation via its activity on several cell types in the vasculature. 5-HT receptors (either 5-HT1-like or not) on endothelial cells mediate the release of EDRF and prostaglandins, which act locally on smooth muscle cells to cause relaxation (Peach et al., 1985; Leff et al., 1987). Stimulation of 5-HT1 receptors on sympathetic nerve terminals inhibits the release of NE, an effect that also reduces vascular tone. Stimulation of 5-HT1-like receptors in the smooth muscle of some vessels may also cause vasodilatation (Feniuk et al., 1983; Bradley et al., 1986; Lee et al., 1994). In the cerebral arteries of the cat and human, the receptor on which 5-HT acts to cause dilatation resembles β-adrenoceptors, because β-adrenoceptor antagonists competitively antagonize both relaxant responses to 5-HT and isoproterenol (Edvinsson et al., 1978).

We were, therefore, interested in the effect of 5-HT on the unusual facial vein. In the present study, special attention was paid to characterize the relaxant effect of 5-HT on the rabbit facial vein to determine whether (a) the endothelium, (b) prostanoids, or (c) β-adrenoceptors were involved in the 5-HT relaxation response.

Materials and Methods

This study was approved by the Animal Welfare Committees of both Hiroshima University School of Medicine and Toho University School of Medicine.

Male Japanese White rabbits, weighing 2–3 kg, were anesthetized with pentobarbital sodium 35 mg/kg, i.v. and exsanguinated from the common carotid artery. The facial vein was dissected and placed in chilled Krebs' bicarbonate solution. The composition of the solution (in mM) was: NaCl, 119; KCl, 4.7; CaCl2, 2.5; KH2PO4, 1.2; MgSO4, 1.2; NaHCO3,
25.0 and glucose, 11.1. The solution was previously aerated with a gas mixture of 95% O₂ and 5% CO₂.

The isolated vein segments were cleaned of connective tissue under a dissecting microscope and made into ring preparations 4 mm long. In some preparations, the endothelium was rubbed gently using a thin wooden stick, and the absence of endothelium was confirmed by the absence of acetylcholine- or a calcium ionophore A23187-induced relaxation (Tsuru et al., 1987). Each preparation was suspended in a 10 ml tissue bath which contained Krebs' solution aerated with the gas mixture and maintained at 37°C, as described previously (Tsuru et al., 1987). Isometric tension was recorded on an ink-writing oscillograph (Nihon Kohden Kogyo, Tokyo, Japan, model WI-641G) via force-displacement transducers (Nihon Kohden Kogyo, model TB-611T). The suspended preparations were equilibrated for 1.5 hr without a load before starting experiments. During the equilibration period, the bath solution was renewed about every 20 min. After being given an optimum load of 0.3 g, the facial vein exhibited sustained intrinsic tone (Pegram et al., 1976; Tsuru et al., 1987). Blood vessel relaxation responses were examined in the preparations which exhibited spontaneous contraction (no constricting agent was used in the present study). The maximum relaxation of each preparation was determined by adding 10⁻⁵ M NE at the end of experiment, and the degree of 5-HT-induced relaxation was expressed as a percentage of the NE-induced maximum relaxation.

To measure responses to electrical stimulation, the venous preparation was sandwiched between a pair of parallel platinum-wire electrodes 1 mm in diameter separated by 1.5 mm. The method of recording changes in tension was the same as stated above. TNS was effected by means of an electronic stimulator (Nihon Kohden Kogyo, model SEN-3301) at approximately 10 min intervals by giving a train of 60 or 120 pulses at each frequency (Tsuru and Uematsu, 1986). The TNS parameters were 0.2 msec duration and a supramaximal intensity of 25 V.

Drugs used were as follows: 5-hydroxytryptamine creatinine sulfate, indomethacin, nor-epinephrine bitartrate, (−)-propranolol hydrochloride, indomethacin, acetylcholine chloride, atropine sulphate, N⁶-nitro-L-arginine, and calcium ionophore A23187 were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Ketanserin, methysergide maleate and SDZ-205, 557 (4-amino-5-chloro-2-methoxy-benzoic acid 2-(diethylamino)-ethylester) hydrochloride were obtained from RBI (Research Biochemicals Incorporated, Natick, MA, U.S.A.).

Statistical analysis: Results shown in the text and figures are expressed as the mean ± S.E.M. obtained from 4 to 6 experiments. Statistical significance of difference between two means was determined by Student's paired t-test. Differences with P values less than 0.05 were considered to be statistically significant.

Results

The buccal portion of the facial vein exhibited intrinsic myogenic tone when it was stretched, as reported previously (Pegram et al., 1976; Winquist and Bevan, 1980; Tsuru et al., 1987), and the contraction persisted for hours. A few preparations which did not exhibit significant myogenic tone were not used in the present study. A typical recording of the response to 5-HT is presented in Fig. 1. In this figure, the preparation was given an initial
Fig. 1. Typical recording of 5-HT-induced relaxation of the rabbit facial vein. The short horizontal bar to the left shows the initial stretched level. The numerals represent negative logarithms of molar concentrations of 5-HT. Triangles show 3-fold increases from previous concentrations. In the subsequent figures, 5-HT concentration-response curves were obtained by administering 5-HT in 10-fold steps. NE-5 (□): norepinephrine 10^{-5} M.

stretch of 0.3 g (shown by the short horizontal bar just before the recording), and the myogenic tone reached a plateau of about 0.66 g. 5-HT concentration-dependently relaxed facial veins exhibiting myogenic tone. After observation of 5-HT relaxation response, NE 10^{-5} M was administered to obtain maximal relaxation, and the degree of 5-HT-induced relaxation was calculated as a percentage of NE-induced maximum relaxation.

There was no significant difference in the development of myogenic tone between control and endothelium-rubbed preparations, and the relaxant response to 5-HT was not affected significantly by rubbing the endothelium, as shown in Fig. 2. The relaxant response to 5-HT was also not influenced by 10^{-4} M L-NNA, an inhibitor of NOS, or by 10^{-5} M indomethacin, an

Fig. 2. Effect of rubbing the endothelium on the 5-HT concentration-response curve in the rabbit facial vein. The endothelium was gently rubbed by using a wooden stick. Ordinate represents % of maximum relaxation induced by 10^{-5} M norepinephrine. There was no significant difference in the 5-HT-induced relaxation between the control and endothelium-rubbed preparations. Points and bars represent the mean±S.E.M.; n=5.
5-HT-induced relaxation of facial vein

Fig. 3. Effect of NG-nitro-L-arginine (L-NNA, left panel) and indomethacin (right panel) on the 5-HT concentration-response curves in the rabbit facial vein. There was no significant difference in the 5-HT-induced relaxation between the control (○) and L-NNA (10^-4 M)-treated (●) preparations in the left panel or between the control (○) and indomethacin (10^-5 M)-treated (●) preparations in the right panel. Points and bars represent the mean±S.E.M.; n=6.

inhibitor of cyclooxygenase, as shown in Fig. 3, suggesting that the 5-HT response was not mediated by NO or prostanoids endogenously released from the vessel tissues.

A β-adrenoceptor antagonist, propranolol (10^-6 M), reversed TNS-induced relaxation of the facial vein into contraction (Fig. 4), as reported previously (Winquist and Bevan, 1980; Tsuru et al., 1990). However, 5-HT-induced relaxation was not altered by propranolol, suggesting that the relaxation was not mediated by β-adrenoceptors, or by NE released from the adrenergic nerve terminals distributing in the facial vein.

The relaxation response to 5-HT was remarkably and concentration-dependently inhibited by methysergide, a non-selective (both 5-HT_1-like and 5-HT_2) 5-HT receptor antagonist, as shown in Fig. 5. Although the 5-HT response was apparently shifted to the right in parallel by methysergide 10^-7 M and 10^-6 M, 5-HT-induced maximum relaxation was markedly reduced in the presence of methysergide 10^-6 M.

The 5-HT receptor antagonists for 5-HT_1A and 5-HT_4 receptors (NAN-190 and SDZ-205, 557, respectively) did not change the 5-HT responses, as shown in Fig. 6A and 6C. While the 5-HT_2 receptor antagonist, ketanserin had no effect at a low concentration of 3×10^-7 M, at a higher concentration of 10^-6 M it slightly, but significantly, inhibited the relaxation responses to 5-HT at concentrations lower than 10^-6 M, as shown in Fig. 6B.
Fig. 4. Effects of propranolol on the response to transmural nerve stimulation (upper panel) and on the 5-HT concentration-response curve (lower panel) in the rabbit facial vein. One and 2 Hz of rectangular pulses (0.2 msec duration and 25 V) were applied for 1 min as indicated by horizontal bars. Propranolol $10^{-6} \text{M}$ reversed the relaxant response to TNS into contraction, as shown in the upper panel. In contrast, propranolol did not affect the relaxant response to 5-HT, as shown in the lower panel.

Fig. 5. Effect of methysergide on the 5-HT concentration-response curve in the rabbit facial vein. A non-selective 5-HT receptor antagonist, methysergide, concentration-dependently shifted the concentration-relaxant response curve for 5-HT to the right in parallel. Control (○); pretreated with $10^{-7} \text{M}$ (●) and $10^{-6} \text{M}$ (■) of methysergide. Points and bars represent the mean ± S.E.M.; $n=6$. 
Discussion

The present study demonstrated that the rabbit facial vein exhibits spontaneous myogenic tone when it is mechanically stretched. Therefore, in the present study no vasoconstricting agent was used to observe the vasorelaxant effect of 5-HT.

The inhibition of myogenic tone by 5-HT was not affected by rubbing the endothelium, or by L-NNA or indomethacin, indicating that 5-HT-induced relaxation is not due to EDRF or prostanoid released from the vascular tissues. It should be noted that the vein relaxed to TNS and exogenous norepinephrine (NE), mediated via β-adrenoceptors (Pegram et al., 1976; Mellander et al., 1982; Tsuru and Negita, 1989). 5-HT has been shown to possess indirect sympathetic action by inducing the release of NE from adrenergic nerve endings (Marin et al., 1981). However, while the β-adrenoceptor antagonist propranolol completely reversed the relaxing response to sympathetic nerve stimulation in the present study (Fig. 4), it did not change the 5-HT concentration-response curve (Fig. 4). This suggests that 5-HT-induced relaxation was not due to indirect sympathetic actions. Furthermore, although it was reported that 5-HT decreased cerebral arterial tone in cats by acting on vascular β-adrenoceptors (Edvinsson et al., 1978), this is not the case with the rabbit facial vein, since propranolol did not affect 5-HT-induced relaxation. Consequently, 5-HT appears to relax the rabbit facial vein, acting directly via 5-HT receptors on the vascular smooth muscle cells.

5-HT receptors are found in both the G protein-coupled and transmitter-gated ion channel structural families, and they have been divided into seven distinct families or classes (5-HT₁,

Fig. 6. Effect of NAN-190 (A), ketanserin (B) and SDZ-205,557 (C) on the 5-HT concentration-response curve in the rabbit facial vein. 5-HT₄,₅, and 5-HT₆-receptor antagonists, NAN-190 and SDZ-205,557 both at 10⁻⁶ M (●) did not change the 5-HT concentration-response curve, as shown in A and C, respectively. A 5-HT₃-receptor antagonist, ketanserin, at 10⁻⁶ M (●) but not 3 × 10⁻⁷ M (▲), inhibited the relaxant response to 5-HT as shown in B. Points and bars represent the mean ± S.E.M.; n = 6.
5-HT<sub>2</sub>, 5-HT<sub>3</sub>, 5-HT<sub>4</sub>, 5-HT<sub>6</sub>, 5-HT<sub>7</sub>) according to structural diversity and the preferred transduction mechanism (Martin, 1998).

In the present study, the median effective concentration (EC<sub>50</sub>) of 5-HT required to relax the rabbit facial vein was 1.0 - 6.0 x 10<sup>-7</sup> M in the absence of receptor antagonists or an enzyme inhibitor. This value is comparable to those obtained for the rabbit saphenous vein (4.0 x 10<sup>-7</sup> M) (Martin <i>et al.</i>, 1987) and the porcine cerebral vein (3.0 x 10<sup>-7</sup> M) (Lee <i>et al.</i>, 1994). Ueno <i>et al.</i> (1995) have shown that 5-HT inhibition of spontaneous rhythmic contractions in porcine pial veins is accompanied by an increase in cyclic AMP and suggested that 5-HT inhibition is mediated by 5-HT<sub>1</sub>-like receptors on muscle cells that are, however, pharmacologically different from the known 5-HT<sub>1</sub> receptors. This hypothesis is not consistent with the proposal that the 5-HT<sub>1</sub> receptor class, which comprises five different receptors (5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>1E</sub> and 5-HT<sub>1F</sub>), couples preferentially to G<sub>110</sub> to inhibit cyclic AMP formation (Martin, 1998). Propranolol has been reported to be an antagonist not only for β-adrenoceptor but also for 5-HT<sub>1B</sub> receptor (Schoeffter and Hoyer, 1989). Therefore, it is likely that 5-HT-mediated relaxation does not involve the 5-HT<sub>1B</sub> receptor.

Methysergide concentration-dependently inhibited 5-HT-induced relaxation, an effect which was not surmountable (Fig. 5), indicating that the antagonism was non-competitive (Kenakin, 1987). The degree of the antagonistic effect exerted by methysergide on 5-HT inhibition in the rabbit facial vein was almost comparable to that obtained in the porcine pial vein: 5-HT EC<sub>50</sub> values were increased by methysergide 10<sup>-7</sup> M by 5.3-fold in the former and by 6.3-fold in the latter (Lee <i>et al.</i>, 1994). Methysergide has been shown to be a non-specific (both 5-HT<sub>1</sub> and 5-HT<sub>2</sub> receptors) antagonist (Schoeffter and Hoyer, 1988). Therefore, the need for more selective 5-HT receptor subtype antagonists is clear.

As shown in Fig. 6B, a high concentration of ketanserin, the most popular 5-HT<sub>2</sub> receptor antagonist, somewhat inhibited 5-HT-induced relaxation. The 5-HT<sub>2</sub> receptor class, which comprises the three subtypes 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub> and 5-HT<sub>2C</sub>, couples preferentially to G<sub>q/11</sub>, thereby increasing the hydrolysis of inositol phosphates and elevating cytosolic Ca<sup>2+</sup> (Martin, 1998). This intracellular signal transduction by the 5-HT<sub>2</sub> receptor class indicates that the apparent inhibition of 5-HT response in the facial vein by ketanserin at higher concentration (10<sup>-6</sup> M) might be non-specific, similar to the finding that ketanserin at a concentration of 10<sup>-6</sup> M has been shown to block 5-HT<sub>1C</sub> receptors (Hoyer, 1989).

It has been shown that 5-HT<sub>4</sub> receptors mediate relaxation of not only gastrointestinal smooth muscle (Bockaert <i>et al.</i>, 1992) but also vascular smooth muscle (Cocks and Arnold, 1992), increasing the intracellular cyclic AMP level. However, in the present study, the 5-HT<sub>1</sub> receptor antagonist SDZ 205,557 did not affect the 5-HT relaxation response in the rabbit facial vein (Fig. 6C).

In conclusion, 5-HT induces relaxation of the rabbit facial vein by activating 5-HT receptors on smooth muscle cells, probably 5-HT<sub>1</sub>-like receptors, as suggested by Bradley <i>et al.</i> (1986) and Ueno <i>et al.</i> (1995). Since we do not yet have a highly selective antagonist, the exact receptor type involved in 5-HT-induced relaxation of the facial vein remains to be identified.
References

Bockaert, J., Fozard, J.R., Dumuis, A. and Clarke, D.E. (1992) The 5-HT\textsubscript{4} receptor: a place in the sun. *Trends Pharmacol. Sci.* **13**: 141-145.

Bradley, P.B., Engel, G., Feniuk, W., Fozard, J.R., Humphrey, P.P.A., Middlemiss, D.N., Mylecharane, E.J., Richardson, B.P. and Saxena, P.R. (1986) Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. *Neuropharmacol.* **25**: 563-576.

Chiba, S. and Tsukada, M. (1990) Dominant vasodilator action of norepinephrine in isolated, non-preconstricted simian facial vein. *Jpn. J. Pharmacol.* **53**: 267-270.

Cocks, T.M. and Arnold, P.J. (1992) 5-Hydroxytryptamine (5-HT) mediates potent relaxation in the sheep isolated pulmonary vein via activation of 5-HT\textsubscript{4} receptors. *Br. J. Pharmacol.* **107**: 591-596.

Edvinsson, L., Hardebo, J.E. and Owman, C. (1978) Pharmacological analysis of 5-hydroxytryptamine receptors in isolated intracranial and extracranial vessels of cat and man. *Circ. Res.* **42**: 143-151.

Feniuk, W., Humphrey, P.P.A. and Watts, A.D. (1983) 5-Hydroxytryptamine-induced relaxation of isolated mammalian smooth muscle. *Eur. J. Pharmacol.* **96**: 71-78.

Hollenberg, N.K. (1988) Serotonin and vascular responses. *Annu. Rev. Pharmacol. Toxicol.* **28**: 41-59.

Hoyer, D. (1989) 5-Hydroxytryptamine receptors and effector coupling mechanisms in peripheral tissues. In: Peripheral Actions of 5-Hydroxytryptamine, ed. by J.R. Fozard. Oxford University Press, Oxford, UK, pp. 73-99.

Kenakin, T.P. (1987) Pharmacologic Analysis of Drug-Receptor Interaction. Raven Press, New York.

Lee, T.J.-F., Ueno, M., Sunagane, N. and Sun, M.-H. (1994) Serotonin relaxes porcine pial veins. *Am. J. Physiol.* **266**: H1000-H1006.

Leff, P., Martin, G.R. and Morse, J.M. (1987) Differential classification of vascular smooth muscle and endothelial cell 5-HT receptors by use of tryptamine analogues. *Br. J. Pharmacol.* **91**: 321-331.

Marin, J., Arias, M., Salaices, M., Sanchez, C.F. and Recio, L. (1981) Vasoconstrictor effects of serotonin in the isolated superior mesenteric artery of cat. *Gen. Pharmacol.* **12**: 97-101.

Martin, G.R. (1998) 5-Hydroxytryptamine receptors. In: The IUPHAR Compendium of Receptor Characterization and Classification. ed. by the Committee on Receptor Nomenclature and Drug Classification of the International Union of Pharmacology, IUPHAR Media, London, pp. 167-185.

Martin, G.R., Leff, P., Cambridge, D. and Barrett, V.J. (1987) Comparative analysis of two types of 5-hydroxytryptamine receptor mediating vasorelaxation: differential classification using tryptamines. *Naunyn–Schmiedeberg's Arch. Pharmacol.* **336**: 365-373.

Mellander, S., Andersson, P.O., Afzelius, L.E. and Hellstrand, P. (1982) Neural beta-adrenergic dilatation of the facial vein in man. Possible mechanism in emotional blushing. *Acta Physiol. Scand.* **114**: 393-399.

Peach, M.J., Loeb, A.L., Singer, H.A., and Saye, J. (1985) Endothelium-derived vascular relaxing factor. *Hypertension* **7**, Suppl. I.: 194-1100.

Pegram, B.L., Bevan, R.D. and Bevan, J.A. (1976) Facial vein of the rabbit: Neurogenic vasodilation mediated by β-adrenergic receptors. *Circ. Res.* **39**: 854-860.

Sanders-Bush, E. and Mayer, S.E. (1996) 5-Hydroxytryptamine (serotonin) receptor agonists and antagonists. In Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th ed., ed. by J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon and A.G. Gilman, McGraw-Hill, New York, pp. 249-263.
Schoeffter, P. and Hoyer, D. (1988) Centrally acting hypotensive agent with affinity to 5-HT\textsubscript{1A} binding sites inhibit forskolin-stimulated adenylate cyclase activity in calf hippocampus. *Br. J. Pharmacol.*, **95**: 975-985.

Schoeffter, P. and Hoyer, D. (1989) 5-Hydroxytryptamine 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} receptors mediating inhibition of adenylate cyclase activity. Pharmacological comparison with special reference to the effects of yohimbine, rauwolscine and some beta-adrenoceptor antagonists. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **340**: 285-292.

Tsuru, H., Kohno, S., Iwata, M. and Shigei, T. (1987) Characterization of histamine receptors in isolated rabbit veins. *J. Pharmacol. Exp. Ther.*, **243**: 696-702.

Tsuru, H. and Negita, S. (1989) Heterogeneity of \(\beta\)-adrenoceptor in canine veins: comparison among the facial, portal and saphenous veins. *Jpn. J. Pharmacol.*, **51**: 385-395.

Tsuru, H. and Uematsu, T. (1986) Time course of the development of pre- and post-junctional supersensitivity in the rabbit ear artery after decentralization. *Jpn. J. Pharmacol.*, **40**: 273-282.

Ueno, M., Ishine, T. and Lee, T.J.-F. (1995) A novel 5-HT\textsubscript{1C}–like receptor subtype mediates cAMP synthesis in porcine pial vein. *Am. J. Physiol.*, **268**: H1383-H1489.

Winquist, R.J. and Bevan, J.A. (1980) Temperature sensitivity of tone in the rabbit facial vein: Myogenic mechanism for cranial thermoregulation. *Science*, **207**: 1001-1002.

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