ELSEVIER

European Journal of Pharmacology 358 (1998) 165–168

ejp

Short communication

Inhibition of myogenic tone by mibefradil in rat cerebral arteries

Eugene Lam, Peter Skarsgard, Ismail Laher *

Vancouver Vascular Biology Research Center, Department of Pharmacology and Therapeutics, Faculty of Medicine, 2176 Health Sciences Mall, The University of British Columbia, Vancouver, BC, Canada V6T 1Z3

Received 2 July 1998; revised 10 August 1998; accepted 14 August 1998

Abstract

The inhibitory effects of mibefradil (1.0 nM–1.0 μM), a putative selective inhibitor of T-type Ca^{2+} channels that has peripheral and coronary vasodilating properties with few negative inotropic effects, on pressure-induced vasoconstrictions were compared to depolarization- and [Arg^8] vasopressin-induced tone in isolated middle cerebral arteries of the rat. The concentration–response relationships (IC_{50}) for myogenic tone (70 ± 20 nM), depolarization- (53 ± 9 nM) and vasopressin-induced tone (70 ± 10 nM) were equally inhibited by mibefradil. Pressure-induced responses were consistently inhibited by mibefradil throughout the myogenically active pressure range (20–100 mm Hg). These results demonstrate that mibefradil is nonselective in inhibiting Ca^{2+} dependent cerebral artery tone due to myogenic activation, depolarization or receptor activation.

Keywords: Depolarization-induced tone; Mibefradil; Myogenic tone; Vascular smooth muscle; Vasopressin; Cerebral artery; (Rat)

1. Introduction

Blood flow in the cerebral circulation is regulated mainly by an intrinsic capacity of small arteries and arterioles to vasoconstrict in response to raised intravascular pressure (McCarron et al., 1989). This property is independent of the endothelium which releases nitric oxide basally and can also be induced by flow through the lumen. A novel Ca^{2+} channel antagonist, mibefradil (Ro 40-5967), was developed and is currently under investigation in clinical studies as a potential antihypertensive and anti-ischaemic drug (Clozel et al., 1997). Mibefradil has a favorable cardiovascular profile which includes peripheral and coronary vasodilatation, negative chronotropy, an absence of negative inotropic effects and neurohumoral stimulation, and a long plasma half-life. Many of these effects have been ascribed to the drug's selectivity for T-type Ca^{2+} channels. Electrophysiological studies have shown an inhibitory selectivity of T-type Ca^{2+} channels over the L-type by 10 to 300-fold (Mishra and Hermsmeyer, 1994). Therefore, our goals, in the present study, were to compare the effects of mibefradil on three types of induced tone in pressurized cerebral arteries and to examine the degree of inhibition over a physiologically relevant pressure range.

2. Materials and methods

2.1. Materials

Mibefradil dihydrochloride was received as a gift from F. Hoffmann-La Roche (Basel, Switzerland). [Arg^8]vasopressin (vasopressin) and other chemicals were obtained from Sigma (St. Louis, USA). Both drugs were dissolved in distilled water and aliquoted into individual vials which were frozen until required. The physiological salt solution consisted of (in mM): 118 NaCl, 24.9 $NaHCO_3$, 4.7 KCl, 1.18 KH_2PO_4, 1.17 $MgSO_4$, 1.6 $CaCl_2$, 11.1 glucose, and 0.026 EDTA. A depolarizing high K^+ solution was made identical to the physiological salt solution except that equimolar NaCl and KCl contents were exchanged to yield a final concentration of 42.3 mM K^+. Ca^{2+}-free solution contained 2.0 mM EGTA and no $CaCl_2$. All buffers were adjusted to a pH of 7.4 prior to use.

2.2. Methods

Male Sprague–Dawley rats (200–350 g) were anaesthetized with sodium pentobarbital (65 mg/kg) and admin-

* Corresponding author. Tel.: +1-604-822-5882; Fax: +1-604-822-6012; E-mail: laher@unixg.ubc.ca

0014-2999/98/$19.00
PII: S0014-2999(98)00611-6

istered heparin sodium (Hepalean 500 U/kg), i.p. The rats were then killed by decapitation, and second-order middle cerebral arteries were harvested in ice-cold physiological salt solution. These vessels were cleaned of connective tissue and then quickly transferred to an oxygenated (95% O_2/5% CO_2) experimental chamber. The mounting and measurement of the internal diameters using the pressure myograph were performed as previously described by Skarsgard et al. (1997). The superfusate temperature was maintained at 37°C throughout the experiment.

Artery segments were pressurized at 60 mmHg for 60 min during which time the vessels reliably developed myogenic tone. Following this equilibration period, the pressure was either maintained at 60 mmHg or lowered to 10 mmHg to remove myogenic tone. In the presence of pressure-induced tone, cumulative mibefradil concentration–response curves (1.0 nM–1.0 μM) were constructed. Alternately, with the removal of myogenic tone, tone was induced either by the addition of vasopressin (0.1 μM) or depolarizing high K^+ solution prior to the construction of cumulative mibefradil concentration–response curves.

In another set of arteries, active pressure–diameter relationships (10–100 mmHg) were investigated in the absence and presence of mibefradil (60 nM, IC_{50}). After equilibration, the luminal pressure was lowered to 10 mmHg, and the internal diameters of the vessels were allowed to stabilize. The pressure was then stepped up to 20 mmHg, and again, the vessels were allowed time to achieve a steady state diameter. Transmural pressure was repeatedly increased in 20 mmHg steps to a maximum of 100 mmHg; the pressure at each step was held for at least 5 min. At the end of each experiment, passive pressure–diameter relationships were determined for each vessel at the pressures examined. These relationships were attained by substituting the physiological salt solution with Ca^{2+}-free physiological salt solution.

Vessel diameters were normalized and expressed either as percentage inhibition or constriction using the equations provided below where d_P is the passive diameter and d_A is the active diameter.

$$\text{Percentage Constriction} = (d_P - d_A) \times 100/d_P$$

$$\text{Percentage Inhibition} = 1 - \left[(d_P - d_{A'})/(d_P - d_{A^\circ})\right] \times 100$$

d_{A° and $d_{A'}$ are active diameters in the absence and presence of mibefradil, respectively. Concentration–response relationships were fitted to a sigmoidal dose–response equation using the PRISM2 statistical analysis software package. All data are expressed as mean ± S.E.M. Two-way analysis of variance with repeated measures was used to determine if responses to mibefradil were different between myogenic-, agonist-, or depolarization-induced tone, and mean values were compared using paired Student's *t*-tests. A value of $P < 0.05$ was considered significant.

3. Results

Mibefradil caused concentration-dependent decreases in myogenic tone ($n = 5$), high K^+ depolarizing salt solution-induced constrictions ($n = 4$), and vasopressin-induced constrictions ($n = 6$). Prior to the cumulative addition of mibefradil, the percentage constriction of the pressurized arteries (60 mmHg) was 21 ± 5%. Vessels preconstricted by depolarization or vasopressin had comparable degrees of tone (25 ± 7% and 20 ± 4%, respectively). The fitted median mibefradil inhibitory concentrations for myogenic tone, high K^+ depolarizing salt solution- and vasopressin-induced constrictions were 70 ± 20 nM, 53 ± 8 nM, and 70 ± 10 nM, respectively (Fig. 1). No statistical differences were found in mibefradil (1.0 nM–1.0 μM) inhibition of tone in the three groups of experiments (Fig. 1).

In the pressure–response studies, arteries at 10 mmHg lacked tone either in the absence or presence of mibefradil (60 nM; Fig. 2). However, myogenic tone was increased at 20 mmHg (control = 13 ± 3%, treated = 7 ± 2%; $P < 0.02$) and reached a maximum percentage constriction at 60 mmHg (control = 23 ± 3%, treated = 12 ± 2%; $P < 0.04$). At pressures greater than 60 mmHg, the level of myogenic tone in both control and mibefradil-treated conditions decreased slightly but remained statistically different ($P < 0.05$). The range in which these blood vessels

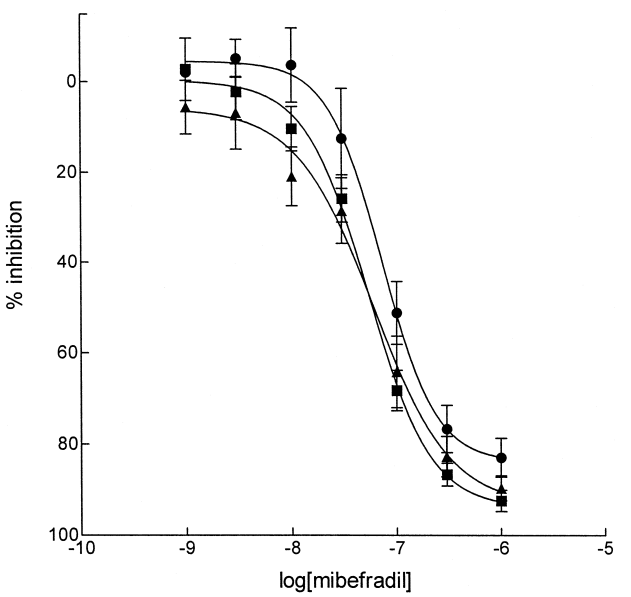

Fig. 1. Inhibition of myogenic, high K^+ depolarizing solution and vasopressin-induced tone in pressurized cerebral arteries. Mibefradil inhibitory concentration–response curves to myogenic tone (triangles), 42.3 mM K^+ physiological salt solution-induced tone (squares) and vasopressin-induced tone (0.1 μM, circles) by mibefradil (1.0 nM–1.0 μM) were nonsignificantly different from each other. The pressure in myogenically active arteries was 60 mmHg, whereas arteries preconstricted with 42.3 mM K^+ or vasopressin were pressurized at 10 mmHg. The data presented as the mean ($n = 4$–6) ± S.E.M. and fitted to a sigmoidal dose–response relationship.

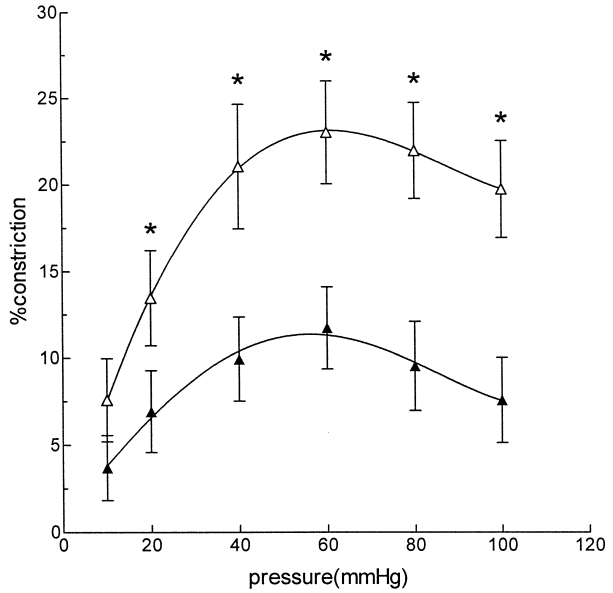

Fig. 2. Inhibition of myogenic tone by mibefradil over a physiological pressure range. Percentage constriction over a pressure range of 10 to 100 mmHg, in the absence (open triangles) or presence of mibefradil (60 nM, closed triangles), was inhibited by the Ca^{2+} channel antagonist. Each point represents the mean ($n = 6$) with the error bars denoting the S.E.M., and the asterisks indicating the pressures at which statistical differences were found ($P < 0.05$).

constricted relative to their passive diameters was relatively constant at pressures of 40–100 mmHg (control = 20–23%, treated = 8–12%).

4. Discussion

This study demonstrates for the first time the inhibitory effect of mibefradil on myogenic tone of small arteries. In addition, the results of this study underscores the importance of extracellular Ca^{2+} and the role of voltage-gated Ca^{2+} channels in the maintenance of tone in these vessels. This importance was affirmed by the indiscriminate inhibition of three examples of induced tone (Fig. 1).

Small arteries and arterioles play a significant role in the regulation of blood flow and resistance; in the cerebral circulation, the contribution of autoregulation to overall vascular tone is higher than in other vascular beds (Meininger and Davis, 1992). Therefore, pressure-induced vasoconstriction or myogenic reactivity is a major component in the control of blood flow. This property of resistance-sized arteries has been shown to be present in most vascular beds of a number of animals, and has only recently been demonstrated in human pial arteries in vitro (Wallis et al., 1997). Thus, the finding that myogenic tone is inhibited by mibefradil is not surprising. Consistent with our observations are mibefradil's antihypertensive effects which have been demonstrated in three rat models of hypertension including genetic, renal, and deoxycorticosterone acetate salt-induced hypertension (Hefti et al., 1990; Menard et al., 1997). Clinical studies have confirmed the dose-dependent blood pressure lowering effects of mibefradil in hypertensive patients (Portegies et al., 1991). The concentration range used in this study lie within clinically relevant plasma concentrations for antihypertensive therapy: a statistically significant decrease in sitting diastolic blood pressure was found in patients with mild to moderate essential hypertension with a single oral dose after 4 weeks (Bernick et al., 1996). The mean trough plasma concentrations for single oral dosages of 25–200 mg daily were 0.15–1.87 μM, which approximately corresponded to our reported IC_{50} of myogenic tone (0.07 ± 0.02 μM). Additionally, Karila-Cohen et al. (1996) demonstrated that coronary flow and diameter in conscious dogs were increased by 103% and 8%, respectively, after intravenous infusion of mibefradil (0.2 mg kg^{-1} min^{-1}).

The ability of small arteries to spontaneously constrict in response to a pressure stimulus independent of neural or humoral influence has been studied for several years. With the development of the pressure myograph, intrinsic factors such as pressure and flow which regulate the diameter of the microvasculature could be examined in vitro. In the presence of the median mibefradil concentration for inhibition (IC_{50}), the percentage constriction was reduced correspondingly which strongly suggests that the level of myogenic activation is dependent on Ca^{2+} flux across the sarcolemma. Our data are in agreement with the well described positive relationship between myogenic reactivity, membrane depolarization, and cytoplasmic Ca^{2+} concentration of resistance arteries (Knot and Nelson, 1998).

Three types of vascular tone were studied: (i) depolarization-induced tone due to voltage-dependent extracellular Ca^{2+} entry, (ii) pressure-induced tone due to a combination of Ca^{2+} entry and changes in intracellular Ca^{2+} sensitivity (Bevan and Laher, 1991) and (iii) receptor-activated tone due to Ca^{2+} release and Ca^{2+} entry. Comparison of the three types of vascular smooth muscle activation revealed nonsignificant differences in the inhibitory potencies of mibefradil, indicating that Ca^{2+} entry through voltage-gated Ca^{2+} channels is a common initiating step. In affirmation, increases in intraluminal pressure were associated with enhanced membrane depolarizations in the rat middle cerebral artery (Knot and Nelson, 1998). At pressures where we observed significant inhibition of constriction (20–100 mmHg) by mibefradil (60 nM, Fig. 2), Knot and Nelson reported depolarizations of 5–30 mV. This magnitude of depolarization reportedly is sufficient to activate T-type Ca^{2+} channels (Perez-Reyes et al., 1998), thus implicating a possible function for these channels in vascular smooth muscle excitation–contraction coupling.

Evidence for the presence of T-type Ca^{2+} channels in vascular smooth muscle is based largely on electrophysiological studies (Mishra and Hermsmeyer, 1994; Clozel et al., 1997). Combined with mibefradil's antihypertensive effects without negative inotropism and direct vasodilating

studies on isolated large vessels (Boulanger et al., 1994; Karila-Cohen et al., 1996), a significant body of evidence is accumulating suggesting the presence and function of these channels in regulating vascular tone. Interestingly, only recently has a neural T-type Ca^{2+} channel been cloned and characterized; Northern blot analysis had revealed a weak transcription in the rat heart (Perez-Reyes et al., 1998). The importance of L-type Ca^{2+} channels, however, cannot be understated since vascular tone can be completely abolished with selective L-type Ca^{2+} channel blockers such as nisoldipine (Knot and Nelson, 1998). Given the indiscriminate block of three types of vascular tone in this study and the reported lack of selectivity of mibefradil, we are uncertain if it's actions are in fact due to nonselective blockade of L-type Ca^{2+} channels. Thus, this relative lack of selectivity of mibefradil for Ca^{2+} channels hinders any understanding of the extent to which particular channels, e.g., T-type channels, may contribute to induced tone.

In summary, pressure-induced tone in the isolated cerebral resistance artery was inhibited by mibefradil at concentrations relevant to antihypertensive therapy. Additionally, depolarization- and agonist-induced tone was also inhibited to a similar extent.

Acknowledgements

This work was supported by funds from the Heart and Stroke Foundation of Canada.

References

Bernick, P.J.L.M., Prager, G., Schelling, A., 1996. Antihypertensive properties of a novel calcium antagonist mibefradil (Ro 40-5967): a new generation of calcium antagonists?. Hypertension 27, 426–432.

Bevan, J.A., Laher, I., 1991. Pressure and flow-dependent vascular tone. FASEB J. 5, 2267–2273.

Boulanger, C.M., Nakashira, M., Olmos, L., Joly, G., Vanhoutte, P.M., 1994. Effects of the Ca^{2+} antagonist Ro 40-5967 on the endothelium dependent response of isolated arteries. J. Cardiovasc. Pharmacol. 23, 869–876.

Clozel, J.P., Ertel, E.A., Ertel, S.I., 1997. Discovery and main pharmacological properties of mibefradil (Ro 40-5967), the first selective T-type calcium channel blocker. J. Hypertens. 1 (Suppl. 5), S17–S25.

Hefti, F., Clozel, J.-P., Osterrieder, W., 1990. Antihypertensive properties of the novel calcium antagonist (1*S*,2*S*)-2-[2[[3-(2-benzimidazolyl) propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphthyl methoxyacetate dihydrochloride in rat models of hypertension: comparison with verapamil. Arzneimittelforschung 40, 417–421.

Karila-Cohen, D., Dubois-Rande, J.-L., Giudicelli, J.-F., Berdeaux, A., 1996. Effects of mibefradil on large and small coronary arteries in conscious dogs: role of vascular endothelium. J. Cardiovasc. Pharmacol. 28, 271–277.

Knot, H.J., Nelson, M.T., 1998. Regulation of arterial diameter and calcium in cerebral arteries of rat by membrane potential and intravascular pressure. J. Physiol. (London) 508, 199–209.

McCarron, J.G., Osol, G., Halpern, W., 1989. Myogenic responses are independent of the endothelium in rat pressurized posterior cerebral arteries. Blood Vessels 26, 315–319.

Meininger, G.A., Davis, M.J., 1992. Cellular mechanisms involved in the vascular myogenic response. Am. J. Physiol. 263, H647–H659.

Menard, J., Karam, H., Veniant, M., Heudes, D., Bruneval, P., Clozel, J.-P., 1997. Effects of calcium blockade on end-organ damage in experimental hypertension. J. Hypertens. 15 (Suppl. 3), S19–S30.

Mishra, S.K., Hermsmeyer, K., 1994. Selective inhibition of T-type Ca^{2+} channels by Ro 40-5967. Circ. Res. 75, 144–148.

Perez-Reyes, E., Cribbs, L.L., Daud, A., Lacerda, A.E., Barclay, J., Williamson, M.P., Fox, M., Rees, M., Lee, J.-H., 1998. Molecular characterization of a neuronal low-voltage-activated T-type calcium channel. Nature 391, 896–899.

Portegies, M.C., Schmitt, R., Kraaij, C.J., Braat, S.H.J.G., Gassner, A., Hagemeijer, F., Poznel, H., Prager, G., Viersma, J.W., van der Wall, E.E., Kleinbloesem, C.H., Lie, K.I., 1991. Lack of inotropic effects of the new calcium antagonist Ro 40-5967 in patients with stable angina pectoris. J. Cardiovasc. Pharmacol. 18, 746–751.

Skarsgard, P., van Breeman, C., Laher, I., 1997. Estrogen regulates myogenic tone in pressurized cerebral arteries by enhanced basal release of nitric oxide. Am. J. Physiol. 273, H2248–H2256.

Wallis, S.J., Firth, J., Dunn, W.R., 1997. Pressure-induced myogenic responses in human isolated cerebral resistance arteries. Stroke 27, 2287–2291.