ELSEVIER

European Journal of Pharmacology 439 (2002) 171–172

ejp

www.elsevier.com/locate/ejphar

Rapid communication

Constitutively active melatonin MT_1 receptors in male rat caudal arteries

Çağatay Erşahin [a,b], Monica I. Masana [a], Margarita L. Dubocovich [a,b,c,*]

[a] *Department of Molecular Pharmacology and Biological Chemistry (S215), Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL, 60611 USA*

[b] *Northwestern Drug Discovery Program, Northwestern University Medical School, Chicago, IL, 60611 USA*

[c] *Department of Psychiatry and Behavioral Sciences, Northwestern University Medical School, Chicago, IL, 60611 USA*

Received 12 February 2002; accepted 15 February 2002

Abstract

This study assessed the state of melatonin MT_1 receptor coupling in sections of male rat caudal arteries by [^{35}S]GTPγS binding autoradiography. The melatonin MT_1 receptor inverse agonist 4-phenyl-2-propionamidotetraline (4P-PDOT) (0.1–1 μM) significantly decreased [^{35}S]GTPγS binding compared to basal, strongly suggesting the presence of constitutively active receptors. Formation of constitutively active receptors during subjective day, when the levels of melatonin are low, may be a physiological mechanism by which the organism maintains vascular tone.

Keywords: Constitutive activity; Melatonin MT_1 receptor; 4P-PDOT (4-phenyl-2-propionamidotetraline)

In the mammalian vasculature, melatonin activates two melatonin receptors, the MT_1 (vasoconstriction) and the MT_2 (vasodilatation) (Doolen et al., 1999). Luzindole or 4-phenyl-2-propionamidotetraline (4P-PDOT) at concentrations known to act as both, melatonin MT_2 receptor competitive antagonists (Dubocovich et al., 1998) and melatonin MT_1 inverse agonists/antagonists (see references in Masana and Dubocovich, 2001) potentiate melatonin-mediated vasoconstriction (Doolen et al., 1999; Krause et al., 2000). This potentiation results in part from blockade of a vasodilatory response mediated by melatonin MT_2 receptors (Doolen et al., 1999); however, an effect of these ligands as inverse agonists on vascular melatonin MT_1 receptors cannot be excluded (Masana and Dubocovich, 2001). Here, we investigated whether melatonin MT_1 receptors in male rat caudal arteries were constitutively active.

Male Fisher 344 rats (150–200 g) were sacrificed by decapitation during the light phase (12/12 light/dark) when blood melatonin is low (Masana and Dubocovich, 2001). Caudal arteries were dissected and sections (20 μm) were prepared for quantitative receptor autoradiography with 2-[^{125}I]iodomelatonin (Dubocovich et al., 1998) or [^{35}S]GTPγS (Sim et al., 1997) as described.

The melatonin MT_1/MT_2 receptor radioligand 2-[^{125}I]iodomelatonin (120 pM) revealed robust specific binding defined by melatonin (1 μM) (3.0 ± 0.5 fmol/mg protein, $n = 12$) in rat caudal artery sections. Specific binding was primarily to melatonin MT_1 receptors, as it was not affected by melatonin MT_2 receptor selective concentrations of 4P-PDOT, as also demonstrated in the suprachiasmatic nucleus (Dubocovich et al., 1998).

Melatonin (0.1–1 μM) stimulated [^{35}S]GTPγS binding over basal (Fig. 1), probably through activation of melatonin MT_1 receptors, since this response was not antagonized by melatonin MT_2 receptor selective concentrations of 4P-PDOT (not shown). Interestingly, 4P-PDOT (Fig. 1) or luzindole (not shown) when used alone at concentrations (0.1 and 1 μM) known to act as melatonin MT_1 receptor inverse agonists (see references in Masana and Dubocovich, 2001) significantly decreased [^{35}S]GTPγS binding to caudal arteries, suggesting the presence of constitutively active receptors. At a higher concentration (10 μM), 4P-PDOT did not affect [^{35}S]GTPγS binding, suggesting an action as partial agonist on melatonin MT_1 and/or MT_2 receptors that counteracts its inverse agonistic effect (Browning et al., 2000; Masana and Dubocovich, 2001).

In rat caudal arteries, a fraction of the melatonin MT_1 receptors in rat caudal arteries appears to exist in a constitutively active form. We conclude that the potentiation of

* Corresponding author. Department of Molecular Pharmacology and Biological Chemistry (S215), Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL, 60611 USA. Tel.: +1-312-503-8005; fax: +1-312-503-2334.

E-mail address: mdubo@northwestern.edu (M.L. Dubocovich).

0014-2999/02/$ - see front matter
PII: S0014-2999(02)01407-3

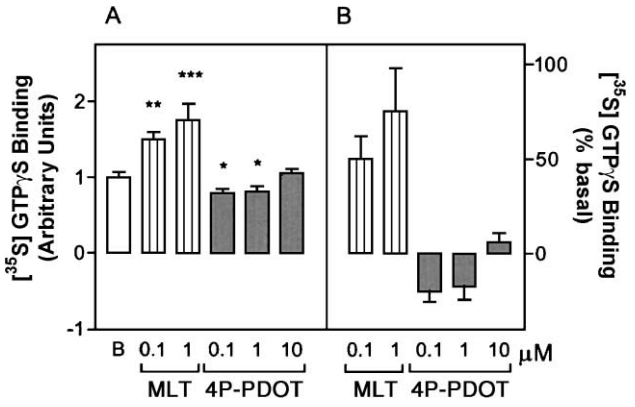

Fig. 1. [^{35}S]GTPγS binding to rat caudal arteries. Sections were incubated at 25 °C in assay buffer (50 mM Tris–HCl, 3 mM $MgCl_2$, 0.2 mM EGTA, 100 mM NaCl, 0.5% bovine serum albumin, pH 7.4) alone for 10 min, and then sequentially with GDP (1 mM) and with GDP (1 mM) and [^{35}S]GTPγS (50 pM) for 30 min each. A. [^{35}S]GTPγS binding was determined in the absence (B) and presence of melatonin (MLT) or 4P-PDOT. B. [^{35}S]GTPγS binding to rat caudal arteries expressed as percent of basal (B). Results are expressed as mean ± S.E.M. ($n=5$) and analyzed variance (ANOVA) repeated measures. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, when compared with basal.

melatonin-mediated vasoconstriction in caudal artery in the presence of 4P-PDOT or luzindole (Doolen et al., 1999; Krause et al., 2000) may involve two independent mechanisms, i.e., selective blockade of melatonin MT_2 receptors mediating vasodilation and shifting constitutively active melatonin MT_1 receptors to the uncoupled form, making more receptors available for constriction.

Acknowledgements

This research was supported by grants USPHS RO1 MH 42922 (M.L.D.) and T32 AG00260 (Ç.E.).

References

Browning, C., Beresford, I., Fraser, N., Giles, H., 2000. Pharmacological characterization of human recombinant melatonin MT_1 and MT_2 receptors. Br. J. Pharmacol. 129, 877–886.

Doolen, S., Krause, D.N., Duckles, S.P., 1999. Estradiol modulates vascular response to melatonin in rat caudal artery. Am. J. Physiol. 276, H1281–H1288.

Dubocovich, M.L., Yun, K., Al-Ghoul, W.M., Benloucif, S., Masana, M.I., 1998. Selective MT_2 melatonin receptor antagonists block melatonin-mediated phase advances of circadian rhythms. FASEB J. 12, 1211–1220.

Krause, D.N., Geary, G.G., Doolen, S., Duckles, S.P., 2000. Melatonin and cardiovascular function. In: Olcese, J. (Ed.), Melatonin after Four Decades: An Assessment of Its Potential. Kluwer Academic Publishing/Plenum Press, NY, pp. 299–310.

Masana, M.I., Dubocovich, M.L., 2001. Melatonin receptor signaling: finding the path through the dark. Sci. STKE. 2001, E39.

Sim, L.J., Selley, D.E., Childers, S.R., 1997. Autoradiographic visualization in brain of receptor-G protein coupling using [^{35}S]GTPγS binding. Methods Mol. Biol. 83, 117–132.