British Journal of Pharmacology (2006) 147, 128–130 © 2006 Nature Publishing Group All rights reserved 0007–1188/06 $30.00
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COMMENTARY

Fading of 5-HT_4 receptor-mediated inotropic responses to 5-hydroxytryptamine is caused by phosphodiesterase activity in porcine atrium

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Inotropic responses to 5-hydroxytryptamine (5-HT) in human and porcine atrium can fade, suggesting 5-HT_4 receptor desensitization. De Maeyer *et al*., however, show in this issue that inhibition of phosphodiesterases with isobutyl-methyl-xanthine prevents fading of 5-HT_4 receptor-mediated responses to 5-HT and the partial agonist prucalopride in porcine atrium.
British Journal of Pharmacology (2006) **147,** 128–130. doi:10.1038/sj.bjp.0706501; published online 5 December 2005

Keywords: Serotonin; 5-hydroxytryptamine; 5-HT_4 receptors; arrhythmias; porcine atrium; human atrium; porcine ventricle; human ventricle; heart failure; phosphodiesterase

5-Hydroxytryptamine (5-HT) is a cardiostimulant in man. 5-HT increases contractility and hastens relaxation of isolated myocardium from right and left atrium. These effects are mediated through 5-HT_4 receptors, and are associated with increases in cyclic AMP and stimulation of cyclic AMP-dependent protein kinase (Kaumann *et al*., 1990, Sanders & Kaumann, 1992). 5-HT also elicits rate-dependent arrhythmias through 5-HT_4 receptors in atrial trabeculae and myocytes (Kaumann & Sanders, 1994; Sanders *et al*., 1995). Chronic treatment with β_1-adrenoceptor-selective blockers enhances both the inotropic and arrhythmic effects of 5-HT (Kaumann & Sanders, 1994; Sanders *et al*., 1995). A remarkably low density of 5-HT_4 receptors ($\sim$4 fmol mg^{-1}) mediates the atrial effects of 5-HT (Kaumann *et al*., 1996). Initial searches for ventricular 5-HT_4 receptors were negative (Jahnel *et al*., 1992; Schoemaker *et al*., 1993). However, ventricular mRNA for 5-$HT_{4(a)}$ and 5-$HT_{4(b)}$ was detected (Bach *et al*., 2001), making the existence of functional 5-HT_4 receptors plausible. The failure to detect ventricular effects of 5-HT could be due to a very low 5-HT_4 receptor density combined with avid hydrolysis of cyclic AMP by phosphodiesterases (PDE). Indeed, in the presence of the nonselective PDE inhibitor isobutyl-methyl-xanthine (IBMX), 5-HT does increase contractile force, hasten relaxation and cause arrhythmic contractions in ventricular trabeculae. These effects are antagonized by the 5-HT_4-selective blocker GR113808, consistent with functional ventricular 5-HT_4 receptors (Brattelid *et al*., 2004). The 5-HT_4 mRNA is increased four-fold in ventricles from failing hearts compared to nonfailing hearts (Brattelid *et al*., 2004).

Porcine atrial and sinoatrial 5-HT_4 receptors have been used as experimental models for human atrial 5-HT_4 receptors. 5-HT produces tachycardia in healthy volunteers (Le Messurier *et al*., 1959), but the nature of the human sinoatrial 5-HT receptors remains unknown. To investigate the properties of sinoatrial 5-HT receptors, spontaneously beating right atrium of new-born piglets were used (Kaumann, 1990). The chronotropic potency and intrinsic activity of 5-HT and 5-carboxamidotryptamine, as well as of the partial agonists renzapride and cisapride, corresponded to a 5-HT_4 receptor profile and agreed quantitatively with the corresponding positive inotropic effects of these ligands on human atrium (Kaumann, 1990, Kaumann *et al*., 1991b). The results make it plausible that human sinoatrial 5-HT receptors are 5-HT_4 receptors, but experiments to prove this (Kaumann & Sanders, 1998) are still pending. 5-HT_4 receptors also mediate the tachycardia evoked by 5-HT and partial agonists in adult pigs (Villalon *et al*., 1991; Parker *et al*., 1995). In addition, functional 5-HT_4 receptors have been identified in paced left atria of new-born piglets (Kaumann *et al*., 1991a; Parker *et al*., 1995) but not in porcine ventricle (Lorrain *et al*., 1992; Schoemaker *et al*., 1992). However, as found in human ventricle, the PDE inhibitor IBMX also uncovers functional 5-HT_4 receptors in porcine ventricle. Interestingly, 5-HT was twice as efficacious and a 15-fold more potent inotropic agonist on ventricular trabeculae from adult pigs than from new-born piglets (Brattelid *et al*., 2004). The age-related difference was mirrored by a greater PKA activation in trabeculae from adult pigs than from new-born piglets (Brattelid *et al*., 2004).

The inotropic effects of 5-HT are more pronounced on atria from adult humans than on atria from new-born piglets (Kaumann & Sanders, 1998). This is in part related to an even lower atrial 5-HT_4 receptor density in newborn piglet ($\sim$0.3 fmol mg protein^{-1}, Kaumann *et al*., 1995) than in adult man. Several other mammalian species, including the rat, do not express at all functional cardiac 5-HT_4 receptors (Kaumann, 1991). However, recent work has demonstrated the appearance of functional 5-HT_4 receptors that mediate increases in contractile force, cyclic AMP levels and relaxation in ventricular papillary muscles from rats with heart failure 6 weeks after coronary artery ligation (Qvigstad *et al*., 2005).

Inotropic responses to 5-HT and 5-HT_4 receptor partial agonists can fade in human and porcine atrial myocardium

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(Kaumann *et al*., 1991b; Sanders & Kaumann, 1992; Parker *et al*., 1995). Fading is usually attributed to desensitization of the receptor system but, in the case of receptors coupled to G_s protein, hydrolysis of cyclic AMP by PDEs can also contribute. For example, fading of the positive inotropic responses to glucagon is reduced by the PDE4-selective inhibitor rolipram in rat ventricle, suggesting involvement of PDE4 (Juan-Fita *et al*., 2004). In a carefully designed study, De Maeyer *et al*. (2006, this issue) report in paced left atria of young pigs that PDE inhibition with IBMX abolishes fading of the positive inotropic responses to 5-HT. The noncumulative administration of the partial agonist prucalopride increased contractile force that faded and cumulatively administered concentrations failed altogether to elicit effects. IBMX potentiated the responses to 5-HT and allowed the determination of cumulative concentration–effect curves for partial agonists without fade. Unlike inotropic responses, chronotropic responses to 5-HT and partial agonists do not fade in spontaneously beating right atria, suggesting that PDEs may not limit the responses through sinoatrial 5-HT_4 receptors. De Maeyer *et al*. found that atrial inotropic responses, in the presence of IBMX, were less marked in new-born piglets than adolescent pigs, as previously reported for ventricle by Brattelid *et al*. (2004).

The work of De Maeyer *et al*. (2006) clearly highlights PDE-evoked reduction of contractile responses, mediated through porcine 5-HT_4 receptors. The increase of the effects of 5-HT by IBMX is, however, not only due to prevention of cyclic AMP hydrolysis upon 5-HT_4 receptor activation but also to the considerable positive and lusitropic effects caused by IBMX. The IBMX-induced cardiostimulation adds to the 5-HT-evoked cardiostimulation and should be subtracted from the combined effects of 5-HT and IBMX, so that the 5-HT_4 receptor component of PDE activity can be assessed. De Maeyer *et al*. (2006) did not address the role of selective PDE isoenzymes. Experiments with inhibitors selective for the two major cardiac isoenzymes, PDE3 and PDE4, should be carried out to unravel which isoenzyme is mainly responsible for the fade. The sinoatrial tachycardia of 5-HT does not appear to fade but PDE activity could still contribute to reduce this response, although probably with a faster time-course than on left atrial myocardium. De Maeyer *et al*. (2006) confirmed the work of Krobert *et al*. (2005) that prucalopride elicits sinoatrial tachycardia in new-born piglets in the absence of IBMX. Krobert *et al*. (2005) found that the cumulative concentration–effect curve of prucalopride for the sinoatrial tachycardia is bell shaped, raising the possibility of a role for PDEs.

Endogenous 5-HT has been proposed to trigger cardiac arrhythmias (Kaumann, 1994; Brattelid *et al*., 2004) and a PDE inhibitor can facilitate arrhythmias and enhance mortality in heart failure (Packer *et al*., 1991). Therefore, the clinical combination of a PDE inhibitor with a 5-HT_4 receptor partial agonist should be used with caution.

References

BACH, T., SYVERSVEEN, T., KVINGEDAL, A.M., KROBERT, K.A., BRATTELID, T., KAUMANN, A.J. & LEVY, F.O. (2001). 5-$HT_{4(a)}$ and 5-$HT_{4(b)}$ receptors have nearly identical pharmacology and are both expressed in human atrium and ventricle. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **363,** 146–160.

BRATTELID, T., QVIGSTAD, E., LYNHAM, J.A., MOLENAAR, P., AASS, H., GEIRAN, O., SKOMEDAL, T., OSNES, J.B., LEVY, F.O. & KAUMANN, A.J. (2004). Functional serotonin 5-HT_4 receptors in porcine and human ventricular myocardium with increased 5-HT_4 mRNA in heart failure. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **370,** 157–166.

DE MAEYER, J.H., STRAETMANN, R., SCHUURKES, J.A.J. & LEFEBVRE, R.A. (2006). Porcine left atrial and sinoatrial 5-HT_4 receptor induced responses: fading of the response and influence of development. *Br. J. Pharmacol*., **147,** 140–157 (this issue).

JAHNEL, U., RUPP, J., ERTL, R. & NAWRATH, H. (1992). Positive inotropic responses to 5-HT in human atrial but not in ventricular heart muscle. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **346,** 482–485.

JUAN-FITA, M.J., VARGAS, M.L., KAUMANN, A.J. & HERNANDEZ CASCALES, J. (2004). Rolipram reduces the inotropic tachyphylaxis of glucagon in rat ventricular myocardium. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **370,** 324–329.

KAUMANN, A.J. (1990). Piglet sinoatrial 5-HT receptors resemble human atrial 5-$HT_{4\text{-like}}$ receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **342,** 619–622.

KAUMANN, A.J. (1991). 5-HT_4-like receptors in mammalian atria. *J.Neural.Transm.,* **34** (Suppl), 195–201.

KAUMANN, A.J. (1994). Do human atrial 5-HT_4 receptors mediate arrhythmias? *Trends Pharmacol. Sci.,* **15,** 451–455.

KAUMANN, A.J., BROWN, A.M. & RAVAL, P. (1991a). Putative 5-$HT_{4\text{-like}}$ receptors in piglet left atrium. *Br. J. Pharmacol.,* **102**, 98P.

KAUMANN, A.J., LYNHAM, J.A. & BROWN, A.M. (1995). Labelling with [^{125}I]-SB 207710 of a small 5-HT_4 receptor population in piglet right atrium: functional relevance. *Br. J. Pharmacol.,* **115,** 933–936.

KAUMANN, A.J., LYNHAM, J.A. & BROWN, A.M. (1996). Comparison of the densities of 5-HT_4 receptors, β_1- and β_2-adrenoceptors in human atrium: functional implications. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **353,** 592–595.

KAUMANN, A.J. & SANDERS, L. (1994). 5-Hydroxytryptamine causes rate-dependent arrhythmias through 5-HT_4 receptors in human atrium: facilitation by chronic β-adrenoceptor blockade. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **349,** 331–337.

KAUMANN, A.J. & SANDERS, L. (1998). 5-Hydroxytryptamine and human heart function: the role of 5-HT_4 receptors. In: *5-HT_4 Receptors in the Brain and Periphery,* ed. Eglen, R.M., pp. 127–148. Berlin: Springer.

KAUMANN, A.J., SANDERS, L., BROWN, A.M., MURRAY, K.J. & BROWN, M.J. (1990). A 5-hydroxytryptamine receptor in human right atrium. *Br. J. Pharmacol.,* **100,** 879–885.

KAUMANN, A.J., SANDERS, L., BROWN, A.M., MURRAY, K.J. & BROWN, M.J. (1991b). A 5-$HT_{4\text{-like}}$ receptor in human atrium. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **344,** 150–159.

KROBERT, K.A., BRATTELID, T., LEVY, F.O. & KAUMANN, A.J. (2005). Prucalopride is a partial agonist through human and porcine atrial 5-HT_4 receptors: comparison with recombinant human 5-HT_4 splice variants. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **371,** 473–479.

LE MESSURIER, D.H., SCHWARTZ, C.J. & WHELAN, R.F. (1959). Cardiovascular effects of intravenous infusions of 5-hydroxytryptamine in man. *Br.J.Pharmacol.,* **14,** 246–250.

LORRAIN, J., GROSSET, A. & CONNOR, E. (1992). 5-HT_4 receptors, present in piglet atria and sensitive to SDZ 205-557, are absent in papillary muscle. *Eur. J. Pharmacol.,* **229,** 105–108.

PACKER, M., CARVER, J.R., RODEHEFFER, R.J., IVANHOE, R.J., DIBIANCO, R., ZELDIS, S.M., HENDRIX, G.H., BOMMER, W.J., ELKAYAM, U., KUKIN, M.L., MALLIS, G.I., SOLLANO, R.N., SHANNON, R.N., TANDON, P.K. & DEMETS, D.L. (1991). Effect of oral milrinone on mortality in severe chronic heart failure. *N. Engl. J. Med.,* **325,** 1468–1475.

PARKER, S.G., TAYLOR, E.M., HAMBURGER, S.A., VIMAL, M. & KAUMANN, A.J. (1995). Blockade of human and porcine myocardial 5-HT_4 receptors by SB 203186. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **335,** 28–35.

QVIGSTAD, E., BRATTELID, T., SJAASTAD, I., ANDRESSEN, K.W., KROBERT, K.A., BIRKELAND, J.A., SEJERSTED, O.M., KAUMANN, A.J., SKOMEDAL, T., OSNES, J.B. & LEVY, F.O. (2005). Appearance of a ventricular 5-HT_4 receptor-mediated inotropic response to serotonin in heart failure. *Cardiovasc. Res.,* **65,** 869–878.

SANDERS, L. & KAUMANN, A.J. (1992). 5-$HT_{4\text{-like}}$ receptors in human left atrium. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **345,** 382–386.

SANDERS, L., LYNHAM, J.A., BOND, B., DEL MONTE, F., HARDING, S.E. & KAUMANN, A.J. (1995). Sensitization of human atrial 5-HT_4 receptors by chronic β-blocker treatment. *Circulation,* **92,** 3536–3539.

SCHOEMAKER, R.G., DU, X.Y., BAX, W.A., BOS, E. & SAXENA, P.R. (1993). 5-Hydroxytryptamine stimulates human isolated atrium but not ventricle. *Eur. J. Pharmacol.,* **230,** 103–105.

SCHOEMAKER, R.G., DU, X.Y., BAX, W.A. & SAXENA, P.R. (1992). 5-Hydroxytryptamine increases contractile force in porcine right atrium but not in left ventricle. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **346,** 486–489.

VILLALON, C.M., DEN BOER, M.O., HEILIGERS, J.P. & SAXENA, P.R. (1991). Further characterization, by use of tryptamine and benzamide derivatives, of the putative 5-HT_4 receptor mediating tachycardia in the pig. *Br. J. Pharmacol.,* **102,** 107–112.

(Received October 14, 2005
Accepted October 18, 2005
Published online 5 December 2005)