ELSEVIER

European Journal of Pharmacology 383 (1999) 53–56

ejp

www.elsevier.nl/locate/ejphar

Short communication

A novel muscarinic M_4 receptor antagonist provides further evidence of an autoreceptor role for the muscarinic M_2 receptor sub-type

Kiyoyuki Kitaichi, Jamie C. Day, Rémi Quirion *

Douglas Hospital Research Centre, Department of Psychiatry, Faculty of Medicine, McGill University, Montreal, Quebec, Canada H4H 1R3

Received 3 June 1999; received in revised form 17 August 1999; accepted 20 August 1999

Abstract

Muscarinic M_2 (AF-DX 384, BIBN-161) and M_4 (PD102807) receptor antagonists were used to investigate the respective roles of these two receptor sub-types in the regulation of acetylcholine release in the rat hippocampus. In vivo dialysis studies revealed that only the muscarinic M_2 receptor antagonists significantly and concentration-dependently facilitate acetylcholine release. The newly developed muscarinic M_4 receptor antagonist was unable to regulate acetylcholine release except at the highest concentration tested. It would thus appear that the muscarinic receptor acting as negative autoreceptor in the rat hippocampus is of the muscarinic M_2 sub-type, the role of the muscarinic M_4 receptor being minimal in this regard.

Keywords: Acetylcholine release; Microdialysis, in vivo; Muscarinic M_2 receptor; PD102807; AF-DX 384; Muscarinic M_4 receptor

1. Introduction

Five muscarinic receptors have been cloned thus far (for a recent review Wess, 1996). All are members of the seven-transmembrane G-protein coupled receptor family with the muscarinic M_1, M_3 and M_5 sub-types linked to $Gq_{/11}$ proteins and the production of inositides, whereas the muscarinic M_2 and M_4 receptors are associated with the G_i proteins and adenylate cyclase activity.

Most of these receptors are widely expressed throughout the body including the brain. However, their respective physiological roles remain to be fully established, primarily because of the limited availability of truly receptor sub-type selective agonists and antagonists. Among the putative roles of the muscarinic receptor sub-groups, an action as inhibitory autoreceptors regulating the release of acetylcholine has been suggested on the basis of anatomical (Levey et al., 1995), pharmacological (Raiteri et al., 1984; Quirion et al., 1995) and behavioral (Quirion et al., 1995) data. However, it is not clear if these autoreceptors are of the muscarinic M_2 (Raiteri et al., 1984; Quirion et al., 1995) or M_4 (McKinney et al., 1993) sub-type because of the lack of selective antagonists to distinguish between these two related receptor sub-types. For example, it has been shown that BIBN-99, a muscarinic M_2 receptor antagonist unable to fully distinguish between muscarinic M_2 and M_4 receptors, is capable of potently reversing cognitive deficits in aged memory impaired rats (Quirion et al., 1995). This information suggests the possibility of targeting muscarinic M_2 receptors as a means of alleviating learning deficits. It is thus of major importance to clearly establish the identity of the muscarinic receptor sub-type on which such antagonists are likely to act, in order to facilitate acetylcholine release and be of potential clinical relevance in diseases associated with altered cognition.

Recently, PD102807 has been developed as one of the very first selective muscarinic M_4 receptor antagonists (Nelson et al, 1996). In the present study, we used this molecule in addition to the purported muscarinic M_2 receptor antagonists AF-DX 384 and BIBN-161 (Doods et al., 1993) to further investigate the exact nature of the autoreceptor sub-type(s) regulating acetylcholine release in the rat hippocampus, as assessed by in vivo dialysis.

* Corresponding author. Douglas Hospital Research Centre, 6875, Blvd. LaSalle, Verdun, Québec, Canada H4H 1R3. Tel.: +1-514-762-3048; fax: +1-514-762-3034; e-mail: mcou@musica.mcgill.ca

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00607-X

2. Materials and methods

2.1. Animals and drugs

Male Sprague–Dawley rats (270–300 g) were purchased from Charles River Canada (St. Constant, Quebec). Animals were kept in a temperature- (22–24°C), humidity (55 ± 5%) and light- (12 h light–dark cycle, lights on at 07:00) regulated room with food and water available ad libitum for at least 3 days before surgery. The procedures involving animals and their care were conducted in accordance with the McGill University Animal Care Committee and the Canadian Council on Animal Care. BIBN-161 and AF-DX 384 were obtained from Dr. H. Doods, Boehringer Ingelheim, Germany, while PD102807 was obtained from Dr. R. Schwarz, Parke-Davis, USA. AF-DX 384 was dissolved in water, whereas BIBN-161 and PD102807 were dissolved in 0.1 N HCl and brought to pH 5–6 with 0.1 N NaOH. These stock solutions were then diluted appropriately in the buffered perfusate (see below).

2.2. Surgery and in vivo dialysis

Animals were anesthetized by intramuscular injection of acepromazine (0.5 mg/kg, Ayerst Laboratories, Montreal, Quebec), xylazine (5 mg/kg, Miles Canada, Etobicoke, Ontario) and ketamine (50 mg/kg, MTC Pharmaceuticals, Cambridge, Ontario). Transverse probes (AN69 hollow fibers, molecular weight cut off $< 60{,}000$, i.d. = 220 μm, o.d. = 310 μm, Hospal-Gambro, St-Leonard, Quebec) were placed in the dorsal hippocampus (3.8 mm posterior to bregma; 3.5 mm ventral to the surface of the skull), according to coordinates obtained from the atlas of Paxinos and Watson (1987).

Two days after surgery, in vivo dialysis was performed to monitor acetylcholine release, as described in detail elsewhere with minor modifications (Kitaichi et al., 1999). Briefly, probes were perfused at a flow rate of 2.34 μl/min with a perfusate containing: NaCl (125 mM), KCl (3 mM), $CaCl_2$ (1.3 mM), $MgCl_2$ (1.0 mM), $NaHCO_3$ (23 mM) in aqueous phosphate buffer (1 mM, pH 7.3), 100 nM neostigmine (RBI, Watick, MA) was included to prevent the degradation of acetylcholine. After a 1 h equilibration period, dialysates were collected every 10 min. Immediately after basal acetylcholine release stabilized, perfusate containing different concentrations of AF-DX 384, BIBN-161 or PD102807 was perfused through the probes until the end of the experiment. The precise location of the probes was verified by standard histological examination.

2.3. High performance liquid chromatography (HPLC) analysis of acetylcholine

The concentration of acetylcholine in dialysates was measured on-line using HPLC coupled with post-column enzymatic reaction and electrochemical detection, as described elsewhere (Day et al., 1998). Acetylcholine release was expressed as a percentage of each animal individual

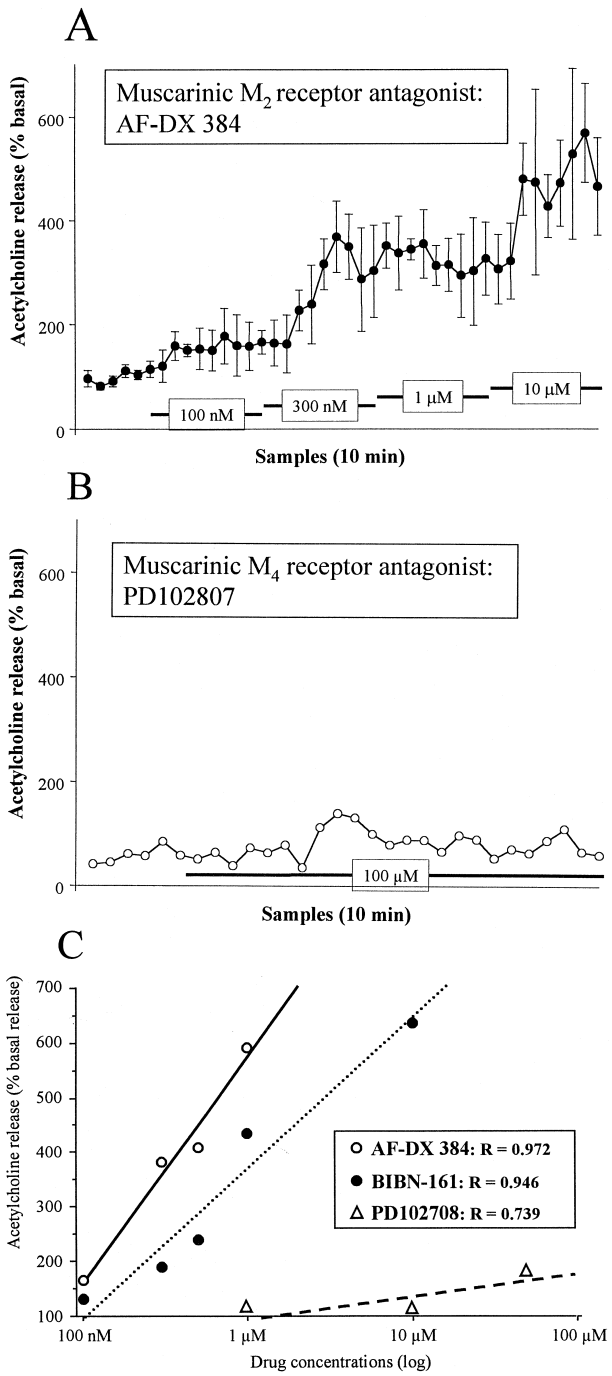

Fig. 1. Effect of muscarinic M_2 and M_4 receptor antagonists, applied locally through the dialysis probe, on hippocampal acetylcholine release assessed by in vivo dialysis and an on-line HPLC-EC assay. (A) Various doses of the muscarinic M_2 receptor antagonist AF-DX 384 increase hippocampal acetylcholine release. Data represent mean ± S.E.M. of six animals. (B) A representative example of the effect of PD102807 (100 μM) in an individual freely-moving rat. The muscarinic M_4 receptor antagonist, administered for up to 4 h, did not increase acetylcholine release. (C) Regression plot analysis of stimulated acetylcholine release as a function of drug concentration. *R* values show the regression values for the individual drugs.

Table 1
Effects of muscarinic M_2 (AF-DX 384, BIBN-151) and M_4 (PD102807) receptor antagonists on hippocampal acetylcholine release
Data represent mean ± S.E.M. of 6–8 determinations at each concentration of the various antagonists. Basal acetylcholine release was 47 ± 7, 45 ± 10 and 47 ± 4 pmol/10 min for the AF-DX384, BIBN-161 and PD102807 groups, respectively. N.D. = not determined.

Acetylcholine release (% basal)			
Concentrations	AF-DX 384 (6)	BIBN-161 (7)	PD102807 (8)
100 nM	166 ± 22[a]	128 ± 22[b]	N.D.
300 nM	247 ± 53[a]	186 ± 58[a]	N.D.
1 μM	325 ± 70[a]	237 ± 29[a]	108 ± 27
10 μM	515 ± 47[a]	430 ± 30[a]	108 ± 2
50 μM	N.D.	632 ± 107[a]	162 ± 20
100 μM	N.D.	N.D.	174 ± 34

[a] $p < 0.001$ vs. basal release.
[b] $p < 0.05$ vs. basal release.

baseline release value (the average amount of acetylcholine in six samples collected immediately preceding drug treatment). All data are expressed as mean ± S.E.M.

3. Results

As shown in Fig. 1A and Table 1, the two muscarinic M_2 receptor antagonists AF-DX 384 and BIBN-161 facilitated acetylcholine release in the rat hippocampus in a concentration dependent manner. Both drugs yielded a significant effect at 100 nM (Table 1) but AF-DX384 was slightly more potent than BIBN-161, as shown in Fig. 1C. Both AF-DX 384 (10 μM) and BIBN-161 (50 μM) were able to potently stimulate acetylcholine release, with 5–6 fold increments in release seen at these concentrations.

In contrast, the purported muscarinic M_4 receptor antagonist PD102807 was unable to modulate release at concentrations lower than 50 μM, with only minimal effects (1.6–1.7-fold over baseline) being observed at very high concentrations (Fig. 1B, Table 1). The lack of significant effects of PD102807 on hippocampal acetylcholine release is also evident in the graphed representation of the regression analysis performed for the three antagonists tested here (Fig. 1C).

4. Discussion

In vivo acetylcholine release in the adult rat hippocampus was clearly stimulated, in a concentration-dependent manner, by AF-DX384 > BIBN-161 ⋙ PD102807. The first two molecules have higher or equal affinities for the cloned muscarinic M_2 than the M_4 receptor sub-types while the reverse is true for the newly developed muscarinic M_4 receptor antagonist PD102807 (Doods et al., 1993; Nelson et al., 1996). In fact, the apparent IC50 (nM) values of AF-DX384 are 325, 16, 150, 10, and 1860 for the M_1, M_2, M_3, M_4 and M_5 sub-type, respectively, and 285, 15, 360, 52, and 220 for BIBN-161. In contrast, PD102807 has its highest affinity for the muscarinic M_4 receptor with IC50 (nM) values of 6600, 3400, 950, 90, and 7400 for the transfected human muscarinic M_1 to M_5 sub-types (Nelson et al., 1996). Taken together, those data support the hypothesis that the muscarinic receptor regulating acetylcholine release is of the M_2 and not the M_4 sub-type, at least in the rat hippocampus.

Earlier anatomical (Levey et al., 1995) and functional (Raiteri et al., 1984; Quirion et al., 1995) data also suggested the preferential role of the muscarinic M_2 receptor as a negative autoreceptor regulating acetylcholine release. However, another study proposed that the M_4 sub-type was in fact acting as the autoreceptor (McKinney et al., 1993). The lack of adequately selective tools to distinguish between muscarinic M_2 and M_4 receptors likely explains this apparent discrepancy.

Recently, we obtained molecular evidence of the role of muscarinic M_2 receptors in the regulation of acetylcholine release (Kitaichi et al., 1999). Using an antisense approach combined with in vivo dialysis, we have shown that a treatment with antisense directed against the muscarinic M_2 receptor blocked the ability of antagonists such as AF-DX 384 to facilitate acetylcholine release, whereas treatment with a specific muscarinic M_4 receptor antisense (or missense oligodeoxynucleotides) failed to alter AF-DX 384's ability to modulate acetylcholine release in the rat hippocampus. Hence, the data reported in the present study are in agreement with those obtained using a molecular approach.

Acknowledgements

This work was supported by an M.R.C. of Canada grant to R. Quirion, a chercheur-boursier of the F.R.S.Q. J. Day holds a fellowship award from the M.R.C.C. K. Kitaichi is now at the Department of Medical Technology, Nagoya University School of Health Sciences, Nagoya, Japan.

References

Day, J.C., Koehl, M., Le Moal, M., Maccari, S., 1998. Corticotropin-releasing factor administered centrally, but not peripherally, stimulates hippocampal acetylcholine release. J. Neurochem. 71, 622–629.

Doods, H.N., Quirion, R., Mihm, G., Engel, W., Rudolf, K., Enzeroth, M., Schiavi, G.B., Ladinsky, H., Bechtel, W., Ensinger, H.A., Mendla, K.D., Eberlein, W., 1993. Therapeutic potential of CNS-active M_2 antagonists: novel structures and pharmacology. Life Sci. 52, 497–503.

Kitaichi, K., Hori, T., Srivastava, L.K., Quirion, R., 1999. Antisense oligodeoxynucleotides against the muscarinic M_2, but not M_4, receptor supports its role as autoreceptors in the rat hippocampus. Mol. Brain Res. 67, 98–106.

Levey, A.I., Edmunds, S.M., Koliastos, V., Wiley, R.G., Heilman, C.J., 1995. Expression of M_1–M_4 muscarinic acetylcholine receptor pro-

teins in rat hippocampus and regulation by cholinergic innervation. Neuroscience 15, 4077–4092.

McKinney, M., Miller, J.H., Aagaard, P.J., 1993. Pharmacological characterization of the rat hippocampal muscarinic autoreceptor. J. Pharmacol. Exp. Ther. 264, 74–78.

Nelson, C.B., Augeli-Szafran, C.E., Schwarz, R.D., Penvose, J.R., Jaen, J.C., Wiley, J., Frey, K.A., 1996. Pharmacological characterization of PD102807: an M_4 selective muscarinic antagonist. Soc. Neurosci. Abstr. 22, 1258.

Paxinos, G., Watson, D., 1987. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Quirion, R., Wilson, A., Rowe, W., Aubert, I., Richard, J., Doods, H., Parent, A., White, N., Meaney, M.J., 1995. Facilitation of acetylcholine release and cognitive performance by an M_2-muscarinic receptor antagonist in aged memory-impaired rats. J. Neurosci. 15, 1455–1462.

Raiteri, M., Leardi, R., Marchi, M., 1984. Heterogeneity of presynaptic muscarinic receptors regulating neurotransmitter release from rat brain. J. Pharmacol. Exp. Ther. 228, 209–214.

Wess, J., 1996. Molecular biology of muscarinic acetylcholine receptors. Crit. Rev. Neurobiol. 10, 69–99.