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Novel persistent activation of muscarinic M_1 receptors by xanomeline

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Abstract

The muscarinic agonists, xanomeline and carbachol, displayed similar intrinsic activities in stimulating neuronal nitric oxide synthase at muscarinic M_1 receptors in Chinese hamster ovary (CHO) cells, with xanomeline being more potent. Pre-incubation (1 h) with 1 μM xanomeline, followed by extensive washing, resulted in a significantly elevated basal response, which was absent on co-incubation with atropine. This phenomenon was not observed with carbachol. This is the first report of a persistent, receptor-activating effect of a muscarinic agonist. © 1997 Elsevier Science B.V.

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The cholinergic hypothesis of dementia postulates a role for the degeneration of basal forebrain cholinergic neurones in the symptomatology of the disorder (Whitehouse et al., 1982). Research efforts have been directed towards replacement of deficient levels of the endogenous neurotransmitter, acetylcholine, or to the targeting of selective agonists to post-synaptic muscarinic M_1 receptors. These receptors are involved in learning and memory, and have been shown to remain relatively unaltered throughout the progression of the disease (Bymaster et al., 1996).

Pre-clinical studies with xanomeline, a novel and potent muscarinic receptor agonist, have identified this compound as displaying a functional selectivity for M_1 receptors (Shannon et al., 1994; Bymaster et al., 1996). Results from clinical trials with this agent have shown significant improvement in cognitive function of Alzheimer's patients (Bodick et al., 1997). Thus, xanomeline remains a promising new drug in the treatment of this disorder.

In order to further probe the molecular nature of both the high potency and functional selectivity of xanomeline, experiments were undertaken utilizing the activation of neuronal nitric oxide synthase as a functional marker of muscarinic M_1 receptor activation in Chinese hamster ovary (CHO) cells.

CHO cells, stably transfected with the human M_1 muscarinic receptor gene (courtesy of Dr. M. Brann, University of Vermont Medical School) and the gene for neuronal NO synthase (courtesy of Dr. S.H. Snyder and Dr. D.R. Bredt, The Johns Hopkins University) were grown to confluence and harvested as described previously (Hu and El-Fakahany, 1990).

The activity of the neuronal NO synthase enzyme was assayed by measuring the conversion of L-[^{3}H]arginine to L-[^{3}H]citrulline, according to the method of Bredt and Snyder (1989) with modifications (Wang et al., 1994). In order to determine concentration–response relationships of agonist-induced activation of neuronal NO synthase, cells (approximately 5×10^5 per tube) were exposed to increasing concentrations of either carbachol or xanomeline for 1 h at 37°C. Both ligands produced a concentration-dependent increase in the formation of L-[^{3}H]citrulline. Non-linear regression analysis of five independent experiments (performed in triplicate) yielded the following logistic parameters: carbachol: $pEC_{50} = 6.04 \pm 0.09$, slope = 1.79 ± 0.38, maximum increase over basal = 6.49 ± 1.12; xanomeline: $pEC_{50} = 8.21 \pm 0.04$, slope = 1.79 ± 0.80, maximum increase over basal = 6.99 ± 0.89. A significant difference was found between the pEC_{50} values ($P < 0.001$; Student's *t*-test, two-tailed). Thus, in this functional assay, both compounds displayed similar intrinsic activities, with xanomeline being markedly more potent than carbachol. This finding is consistent with earlier reports using these two agents in a different functional assay (Shannon et al., 1994).

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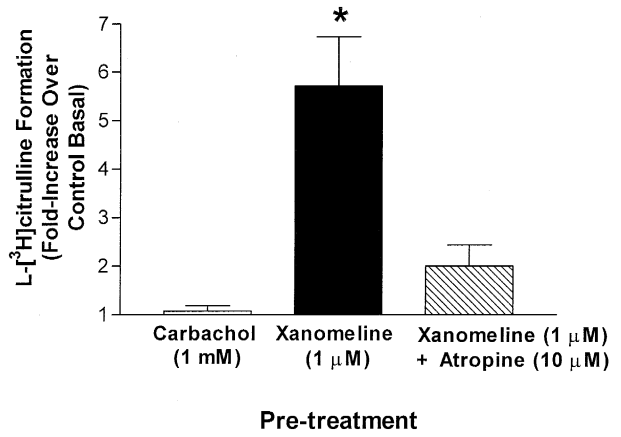

Fig. 1. Effects of pre-treatment with carbachol or xanomeline on basal levels of nitric oxide synthase activity in M_1-CHO cells. Cells were incubated with the indicated concentrations of drugs for 1 h at 37°C before harvesting, washing (3 times) and assaying, as described in the text. Basal enzyme activity was assayed by quantitating the formation of L-[^{3}H]citrulline from L-[^{3}H]arginine over 1 h at 37°C, in the absence of agonist. Bars represent the mean ± S.E.M. of five independent experiments conducted in quintuplicate. Basal activity in control cells was 4090 ± 551 dpm. A one-way analysis of variance, followed by a Bonferroni test, revealed a significant difference ($P < 0.01$) between the cell group treated with xanomeline alone (*) compared to all other groups.

In a parallel series of experiments, cells were incubated for 1 h at 37°C with either 1 mM carbachol, 1 μM xanomeline or 1 μM xanomeline plus 10 μM atropine, prior to harvesting. After harvesting, cells were washed three times with a HEPES-based buffer (Wang et al., 1994) and utilized in experiments quantitating basal neuronal NO synthase activity. Agonist pre-incubation concentrations were chosen on the basis of preliminary binding experiments (not shown) and represent approximately 100 $\times K_D$ concentrations of each ligand.

A striking difference was observed when the cells were pre-incubated with xanomeline, compared to carbachol. Surprisingly, xanomeline pre-treated cells displayed a marked increase in basal L-[^{3}H]citrulline formation (Fig. 1), after extensive washing of free agonist. This increase was almost equal to the maximum possible drug-induced response, as determined above. Pre-incubation with the muscarinic receptor antagonist, atropine, together with xanomeline, prevented this effect (Fig. 1), confirming that the phenomenon was, indeed, mediated by muscarinic receptors.

This result suggests that xanomeline may persistently bind to and activate the muscarinic M_1 receptor under conditions where carbachol has, presumably, been removed. Furthermore, the lack of the persistent xanomeline effect on co-incubating the cells with this agent together with atropine, suggests that the mechanism underlying the phenomenon may involve attachment points on the receptor that are utilized by both ligands.

To our knowledge, this is the first report of a persistent, receptor-activating effect of a muscarinic agonist, under conditions where a conventional agonist exhibits no such response. This finding is significant, both in terms of the treatment of Alzheimer's disease and the understanding of muscarinic acetylcholine receptor function. Experiments aimed at further delineating the mechanism(s) of action of xanomeline at the molecular level are currently in progress.

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References

Bodick, N.C., Offen, W.W., Levey, A.I., Cutler, N.C., Gauthier, S.G., Satlin, A., Shannon, H.E., Tollefson, G.D., Rasmussen, K., Bymaster, F.P., Hurley, D.J., Potter, W.Z., Paul, S.M., 1997. Effects of xanomeline, a selective muscarinic receptor agonist, on cognitive function and behavioural symptoms in Alzheimer's disease. Arch. Neurol. 54, 465.

Bredt, D.S., Snyder, S.H., 1989. Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum. Proc. Natl. Acad. Sci. USA 86, 9030.

Bymaster, F.P., Shannon, H.E., Mitch, C.H., DeLapp, N., Ward, J.S., Calligaro, D.O., Bodick, N.C., Farde, L., Sheardown, M.J., Olesen, P.H., Suzdak, P.D., Swedberg, M.D.B., Sauerberg, P., 1996. Xanomeline: Preclinical and clinical pharmacology of an M1 agonist. In: Fisher, A. (Ed.), Muscarinic Agonists and the Treatment of Alzheimer's Disease. R.G. Landes Company, p. 155.

Hu, J., El-Fakahany, E.E., 1990. Selectivity of McN-A-343 in stimulating phosphoinositide hydrolysis mediated by M_1 muscarinic receptors. Mol. Pharmacol. 328, 895.

Shannon, H.E., Bymaster, F.P., Calligaro, D.O., Greenwood, B., Mitch, C.H., Sawyer, B.D., Ward, J.S., Wong, D.T., Olesen, P.H., Sheardown, M.J., Swedberg, M.D.B., Suzdak, P.D., Sauerberg, P., 1994. Xanomeline: A novel muscarinic receptor agonist with functional selectivity for M_1 receptors. J. Pharmacol. Exp. Ther. 269, 271.

Wang, S.Z., Zhu, S.Z., El-Fakahany, E.E., 1994. Efficient coupling of m5 muscarinic acetylcholine receptors to activation of nitric oxide synthase. J. Pharmacol. Exp. Ther. 268, 552.

Whitehouse, P.J., Price, D.L., Struble, R.G., Clark, A.W., Coyle, J.T., Delong, M.R., 1982. Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. Science (Washington DC) 215, 1237.