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Short communication

Effects of extracellular acetylcholine on muscarinic receptor binding assessed by [^{125}I]dexetimide and a simple probe

Patricia M. Sánchez-Roa [a], Henry N. Wagner Jr. [a,*], Victor L. Villemagne [b], Edythe D. London [b], John R. Lever [a]

[a] *Department of Environmental Health Sciences, Divisions of Nuclear Medicine and Radiation Health Sciences, The Johns Hopkins University, 615 North Wolfe Street, Room 2001, Baltimore, MD, 21205-2179, USA*

[b] *Brain Imaging Center, National Institute on Drug Abuse (NIDA), National Institutes of Health (NIH), Baltimore, MD, 21224, USA*

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Abstract

New pharmacologic approaches to enhance brain cholinergic function focus on increasing intrasynaptic acetylcholine. We examined the usefulness of a simple probe and [^{125}I]dexetimide to evaluate in vivo the effects of extracellular acetylcholine on muscarinic receptor binding in the mouse brain. After radiotracer injection continuous time/activity curves were generated over 330 min. [^{125}I]Dexetimide reached a plateau at 90 min post-injection. To increase extracellular acetylcholine, the anticholinesterase physostigmine was administered at 120 min, producing a reversible decrease in [^{125}I]dexetimide specific binding (23%) for 30 min. These findings demonstrate that dynamic changes in extracellular acetylcholine can be evaluated by displacement of [^{125}I]dexetimide binding in vivo using a simple probe system.

Keywords: Acetylcholine; Muscarinic receptor; Dexetimide; Physostigmine; Brain; Radiation detection system

1. Introduction

Cholinergic receptors, acetylcholine and cholinesterase are widely expressed in the mammalian brain. Central cholinergic pathways are involved in higher cognitive processes such as learning and memory (Jerusalinsky et al., 1997), and their loss or atrophy has been implicated in a variety of neurodegenerative disorders such as senile dementia of the Alzheimer type, Huntington's and Parkinson's diseases (Palacios et al., 1990). Furthermore, the tonic presence of acetylcholine in the neocortex is essential for restoration of cognitive function after basal forebrain damage even if another neurotransmitter or neuroanatomical system is involved in the lesion-induced deficit (Björklund and Dunnett, 1995; Winkler et al., 1995).

Currently, anticholinesterase derivatives (Dawson and Iversen, 1993), cholinergic receptor agonists (Tariot et al., 1988), enhancers of acetylcholine release (Cook et al., 1990) and centrally acting cholinergic channel activators (Buccafusco et al., 1995) are the focus of intensive clinical and experimental trials. These compounds are proposed as potential therapies for senile dementia of the Alzheimer type, where post-mortem loss of cholinergic neurons has been correlated with the memory impairment suffered by these patients (Collerton, 1986). Increasing the intrasynaptic levels of acetylcholine is one of the goals pursued with these pharmacological agents (Schorderet, 1995).

Radioiodinated dexetimide, a muscarinic antagonist, was formerly used for non-invasive assessment of binding to cerebral muscarinic receptors with SPECT in the human brain (Müller-Gartner et al., 1992, 1993; Claus et al., 1997), and with a simple radiation detection system (probe) in small animals (Sasaki et al., 1993). However, the in vivo effect of changes in extracellular acetylcholine on muscarinic receptor binding has not been studied yet with these methods. The purpose of the present study was to determine whether these changes could be evaluated in vivo in the mouse brain following physostigmine administration, using [^{125}I]dexetimide and a probe system.

2. Materials and methods

Twelve male CD-1 mice (Charles River, Wilmington, MA, USA) weighing 28 ± 4 g were assigned to three

* Corresponding author. Tel.: +1-410-955-3680; Fax: +1-410-955-6222.

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groups, four mice each, to monitor the time-course of radiotracer in brain as follows: one control group received [^{125}I]dexetimide and saline, a second control group received [^{125}I]levetimide, and a third group received [^{125}I]dexetimide plus physostigmine. Radioligands were synthesized at high specific activity (1100 mCi μmol^{-1}) as previously described (Wilson et al., 1989). [^{125}I]Levetimide, the inactive enantiomer of [^{125}I]dexetimide, measured non-specific binding (Wilson et al., 1989; Matsumura et al., 1991). Specific binding was defined as the difference between the [^{125}I]dexetimide (total binding) and [^{125}I]levetimide studies. Animals were anesthetized with urethane (1.8 g kg^{-1}, i.p.) 30 min prior to injection of the radiotracer and reanesthetized when necessary.

Brain radioactivity was measured with a multi-channel analyzer based single-crystal radiation detection system (Capintec, Ramsey, NJ, USA), consisting of a cylindric NaI crystal (5 cm × 5 cm × 5 cm), that was calibrated with a ^{137}Cs source before each experiment. A single-parallel hole (5 mm diameter), 30 mm thick, lead collimator was positioned over the head of the mouse centered over the canthomeatal line, 5 mm behind the external angle of the eye, according to the method described by Sasaki et al. (1993) (Fig. 1).

Either [^{125}I]dexetimide or [^{125}I]levetimide, 35.4 ± 7.9 μCi (0.73 ± 0.08 μg kg^{-1}), was injected into the tail vein of each animal. Animals that received [^{125}I]dexetimide were also given either 0.9% sodium chloride or physostigmine (Sigma, St. Louis, MO, USA) in saline (0.5 mg kg^{-1}, i.p.), 2 h after injection of the radiotracer.

Serial, 2-min acquisitions were obtained over a period of 330 min. Measured radioactivity was normalized to injected dose and body weight, and was expressed as cpm μCi^{-1} g^{-1}. All normalized values from each of the [^{125}I]dexetimide and [^{125}I]levetimide experiments were fitted to triple exponential and logarithmic functions, respectively. Fitted values for each [^{125}I]dexetimide and [^{125}I]levetimide experiment and the normalized values for each [^{125}I]dexetimide + physostigmine experiment were averaged for 10-min periods.

Within each group, data were evaluated by one-way analysis of variance (ANOVA) across time. Between group data were evaluated by one-way ANOVA at specific time points after drug injection. When significant F-values were obtained, Dunnet's post-hoc tests were performed to compare group means with control values. $P < 0.05$ was chosen as the minimum criterion for statistical significance.

3. Results

Radioactivity increased progressively after injection of [^{125}I]dexetimide ($n = 4$), reaching a plateau at about 90 min post-injection that lasted until the end of the experiment. Radioactivity after injection of [^{125}I]levetimide

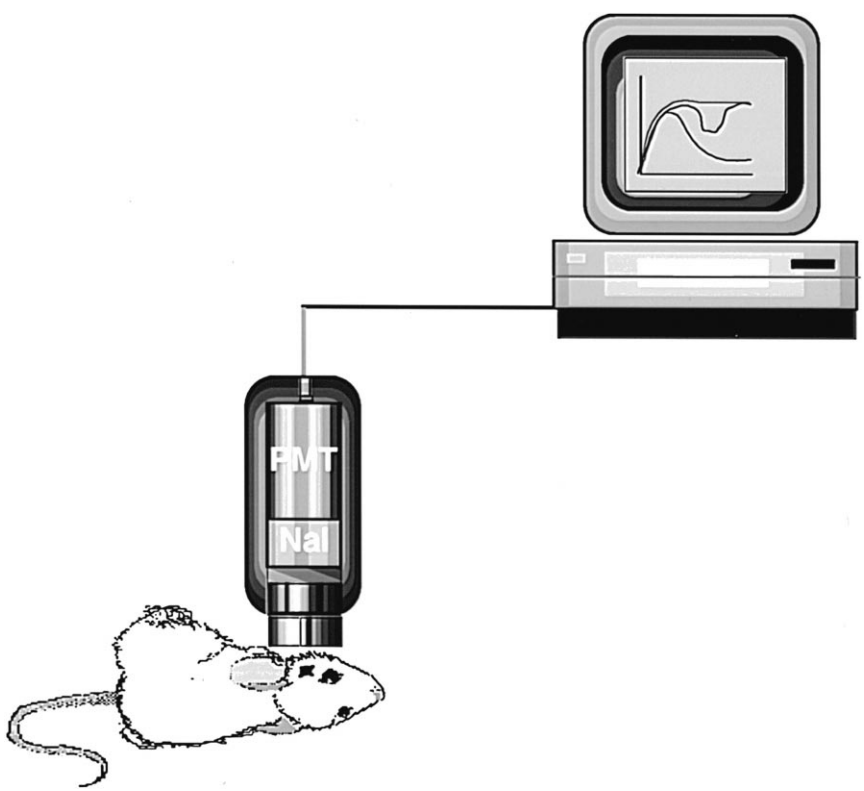

Fig. 1. Schematical representation of the arrangement of a collimated scintillation crystal used for detecting 35 keV gamma rays from the iodinated ligand in the mouse brain. Abbreviations: NaI = sodium iodide (crystal); PMT = photomultiplier tube.

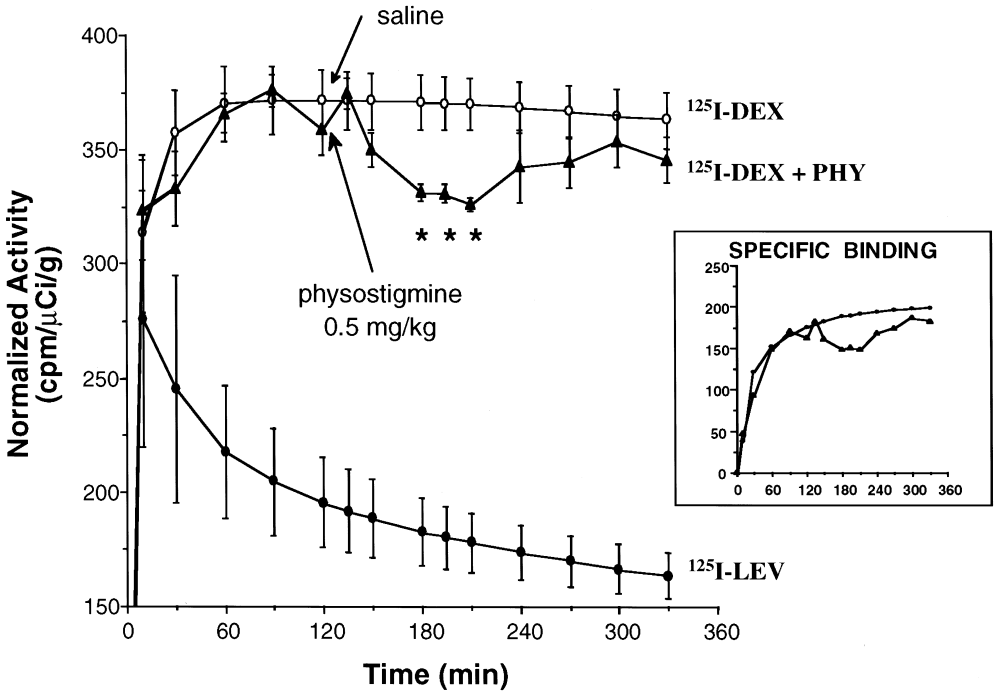

Fig. 2. Time–radioactivity curves obtained after administration of [^{125}I]dexetimide (○, $n = 4$) and [^{125}I]levetimide (●, $n = 4$) to controls. Displacement studies were performed by administration of physostigmine (0.5 mg kg^{-1}, i.p.), 120 min after the injection of [^{125}I]dexetimide (▲, $n = 4$, $* P < 0.05$). Inset shows specific binding ([^{125}I]dexetimide minus [^{125}I]levetimide) in the control (●, $n = 4$) and physostigmine (▲, $n = 4$) groups.

($n = 4$) decreased in a logarithmic fashion after the initial peak, reaching ~ 50% of its initial value by the end of the experiments (Fig. 2).

Administration of physostigmine 120 min after tracer injection ($n = 4$) produced a gradual and significant decrease of [^{125}I]dexetimide binding ($F = 5.05$, $P < 0.0001$). This effect was greatest at 60 min after the injection of physostigmine, and persisted for about 30 min. This decrement represented a 12% decrease in total binding and a 23% decrease in specific binding (Fig. 2, inset), and was significant with respect to saline-treated controls at 180 ($F = 17.95$, $P < 0.0006$), 195 ($F = 15.85$, $P < 0.0016$), and 210 min ($F = 25.02$, $P < 0.0002$) after radiotracer injection. Radioactivity began to return to control values by 90 min after the injection of physostigmine; by 120 min, the difference between the two studies became statistically non-significant ($F = 1.66$, $P > 0.25$) (Fig. 2). All the animals presented transient signs of peripheral parasympathetic cholinergic effects (relaxation of sphincters, increased salivation) approximately 15 min after the administration of physostigmine.

4. Discussion

Physostigmine is a short-acting, reversible cholinesterase inhibitor that increases the synaptic levels of endogenous acetylcholine by inhibiting its enzymatic degradation (Taylor, 1990). In the present study, the sequence of effects of physostigmine on [^{125}I]dexetimide binding can be best described in three phases: a first phase of dissociation of [^{125}I]dexetimide, a second phase of maximal effect of physostigmine, and a third phase of re-association of [^{125}I]dexetimide.

Due to its lipophilicity and low binding to plasma proteins (Somani et al., 1991), physostigmine readily crosses the blood–brain barrier. Bertrand and Beley (1990) reported that 0.5 mg kg^{-1} of physostigmine injected i.p. in unanesthetized mice 15 min prior to killing, produced an 84% increase in endogenous brain acetylcholine as determined by high pressure liquid chromatography. Similar results were obtained from microdialysis experiments in unanesthetized rats 20 min after systemic injection of the same dose of physostigmine, yielding a 40% increase in striatal acetylcholine (Tsai et al., 1996). Other studies reported higher increases of extracellular acetylcholine after physostigmine administration (Messamore et al., 1993a,b). The discrepancies may reflect differences in the treatment regimens and the presence of cholinesterase inhibitors in the perfusate (Messamore et al., 1993a).

In the present study, the action of physostigmine was evidenced by a decrease in radioactivity levels for a period of 60 min (phase 1), probably reflecting displacement of [^{125}I]dexetimide from muscarinic receptors by increased extracellular acetylcholine. Differences in kinetics between the time needed to reach a maximal effect of physostigmine in other experiments (Bertrand and Beley, 1990; Tsai et al., 1996), and the time needed to reach maximal

dissociation of [^{125}I]dexetimide in the present report, may reflect the fact that acetylcholine has a much lower affinity ($IC_{50} = 2 \times 10^{-4}$ M) for brain muscarinic cholinergic receptors than either dexetimide ($IC_{50} = 2.8 \times 10^{-9}$ M) (Laduron et al., 1979) or [^{125}I]dexetimide ($IC_{50} = 5.8 \times 10^{-9}$ M) (Wilson et al., 1989), making it necessary to accumulate larger amounts of acetylcholine to displace the radiotracer effectively from muscarinic binding sites. Though physostigmine was shown to activate nicotinic receptors through a binding site distinct from that of acetylcholine (Pereira et al., 1993), it did not show any significant binding to muscarinic receptors (Xiao et al., 1993). Thus, any effect observed on the time-course of [^{125}I]dexetimide is not related to a direct interaction of physostigmine with muscarinic receptors, but more likely secondary to increased levels of extracellular acetylcholine due to cholinesterase inhibition.

In the second phase (60–90 min period after physostigmine administration), brain radioactivity was significantly reduced by 12%. This reduction was translated into a 23% decrease in specific binding. Maximal levels of acetylcholine after a similar systemic dose of physostigmine were also found to persist for about 30 min in microdialysis experiments (Messamore et al., 1993a,b; Tsai et al., 1996). Although muscarinic antagonists are known to increase the levels of acetylcholine in the presence of an cholinesterase inhibitor (Moor et al., 1998), it is very unlikely that this response was amplified or affected by interaction of the radiolabelled antagonist with muscarinic autoreceptors, since [^{125}I]dexetimide was used at tracer doses that would not to produce any pharmacological effect. Somani and Khalique (1987) reported that the half-life of [^{3}H]physostigmine in rat brain after systemic administration was 11 min, and that the plasma clearance half-time was 15 min. However, the half-time of inhibition of brain cholinesterase was 26 min, indicating that the pharmacodynamic effect of physostigmine was about 2.4 times longer than expected from its pharmacokinetic characteristics. Similar recovery rates for rat brain cholinesterase were reported by Hallak and Giacobini (1986) after i.m. administration of physostigmine. Therefore, the duration of the maximal displacement of [^{125}I]dexetimide by endogenous acetylcholine in these experiments (30 min) is correlated better with the half-time of inhibition of cholinesterase than with the half-life of physostigmine in brain, in agreement with reported decarbamylation rates of cholinesterase after physostigmine administration as mentioned above. Lack of correlation between plasma clearances half-times (pharmacokinetics) and brain receptor occupancy (pharmacodynamics) by a drug has also been demonstrated in vivo in humans with a dual radiation detector probe system (Lee et al., 1988; Kim et al., 1997).

In the present study, normalized radioactivity levels began to return to control values by 90 min after the administration of physostigmine, representing a third phase of re-association of [^{125}I]dexetimide. The difference between [^{125}I]dexetimide–saline and [^{125}I]dexetimide–physostigmine studies became statistically non-significant by 120 min after injection of physostigmine. These results are in agreement with those reported by Hallak and Giacobini (1986), Tsai et al. (1996) and Messamore et al. (1993a,b). It is possible that a muscarinic autoreceptor-mediated autoinhibition of acetylcholine release secondary to cholinesterase inhibition, plays a role during this phase, as was demonstrated in microdialysis experiments (Moor et al., 1998).

Finally, these experiments demonstrate that brain neurotransmitter systems and drugs can be easily studied in vivo with simple radiation detection systems when appropriate radiotracers are available (Sasaki et al., 1993; Liu et al., 1997; Mochizuki et al., 1997). Although results from human studies with [^{123}I]dexetimide and a simple probe are not available yet, the probe system may have certain advantages over SPECT, particularly when therapy follow-up is considered. This is due to its higher temporal resolution, lower cost and better dosimetry characteristics, that permit multiple studies to be performed in the same patient with lower radiation exposure. Our preliminary results suggest that extracellular changes in acetylcholine and the effects of drugs such as physostigmine, can be evaluated by reduction of [^{125}I]dexetimide muscarinic receptor binding and a simple probe. The probe system is a valuable and inexpensive way to examine in vivo the dynamic effects of pharmacological perturbations on neurotransmitter systems. The assessment of the suitability of this method for evaluation of these parameters in humans is therefore warranted.

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