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Parasympathetic excitation of sympathetic innervation after cholinesterase inhibition

Curtis L. Beauregard & [1]Peter G. Smith

Department of Physiology, University of Kansas Medical Center, 3901 Rainbow Blvd. Kansas City, KS 66160-7401, U.S.A.

1 Orbital parasympathetic innervation normally provides prejunctional muscarinic inhibition of sympathetic neurotransmission without activation of excitatory muscarinic receptors located on the innervated smooth muscle. The present study examines the role of acetylcholinesterase (AChE) in limiting the effects of parasympathetically released acetycholine to prejunctional receptors.

2 Urethane anaesthetized rats were placed in a stereotaxic frame, and parasympathetic activation was achieved by electrical stimulation (20 Hz, <2.0 V) of the ipsilateral superior salivatory nucleus. Drugs were administered through a femoral venous cannula. Superior tarsal smooth muscle responses were measured by recording eyelid tension.

3 Parasympathetic stimulation alone caused a small decrease in resting tension; previous studies have shown this to be attributable to attenuation of resting sympathetic tone. Parasympathetic activation following physostigmine administration, however, evoked a large contractile response. Contractions were resistant to atropine but were blocked by gallamine, guanethidine, and phentolamine.

4 We conclude that AChE inhibition results in conversion of orbital parasympathetic nerve function from inhibition of sympathetic neurotransmission to smooth muscle excitation. This occurs as a result of cholinergic activation of excitatory nicotinic receptors on sympathetic varicosities, which elicit the release of noradrenaline.

Keywords: Acetylcholinesterase; autonomic neurotransmission; nicotinic receptors; muscarinic receptors; smooth muscle; prejunctional

Introduction

The sympathetic and parasympathetic divisions of the autonomic nervous system interact to control activity of visceral and glandular targets. This interaction occurs both postjunctionally through receptors located on the target organs (Westfall, 1980) and prejunctionally via receptors on the nerves themselves (Starke, 1981). While the classical view has held that transmitters released from autonomic varicosities diffuse throughout the extracellular space to activate both pre- and post-junctional receptors indiscriminately (Brock & Cunnane, 1992) more recent evidence suggests this is not always the case (Hirst *et al.*, 1992). In rat periorbital smooth muscle, for example, parasympathetic stimulation causes significant prejunctional inhibition of sympathetically evoked contractions without activating excitatory muscarinic receptors on the smooth muscle (Beauregard & Smith, 1994). Thus, in at least some instances, autonomic neurotransmission may be highly specific, activating restricted populations of receptors.

This activation of specific receptor populations can be altered by changes in the nerve terminal microenvironment. Five weeks following sympathetic denervation, parasympathetic stimulation results in muscarinic excitation of periorbital smooth muscle, instead of the normal attenuation (Smith & Beauregard, 1993). This conversion to excitation is not accompanied by enhanced smooth muscle responsiveness to muscarinic agonists. Therefore, conversion cannot be attributed to postjunctional changes in receptors.

One possible factor contributing to cholinergic excitation may be decreased degradation of acetylcholine (ACh) following sympathectomy. Because sympathetic axons contain substantial amounts of acetylcholinesterase (Silver, 1974; Nyquist-Battie & Moran, 1990), Wallerian degeneration of these axons may result in increased quantities of ACh reaching the smooth muscle to activate excitatory muscarinic receptors. Indeed, a 39% reduction in the density of orbital AChE-positive nerves has been reported 7 days after surgical removal of the superior cervical ganglion (Sharp & Smith, 1992). Therefore decreased breakdown of ACh may be expected following sympathectomy.

To determine if reduced degradation of ACh can account for the parasympathetically-mediated excitation of peri-orbital smooth muscle following sympathectomy, we administered the AChE inhibitor physostigmine and measured smooth muscle tone during activation of the parasympathetic innervation to the rat orbit. If decreased ACh degradation is directly responsible for functional conversion following sympathectomy, then pharmacological inhibition of AChE should also result in muscarinic smooth muscle contraction during parasympathetic activation.

Methods

Experiments were conducted on a total of 16 adult, female, Sprague-Dawley rats (Harlan) weighing 270 to 320 g. Rats were anaesthetized with urethane (1.25 g kg^{-1}, i.p.); adequate anaesthesia was indicated by the absence of deep tendon and corneal reflexes. Rectal temperature was maintained at 36°C. A venous cannula was inserted into a femoral vein, and the animal was placed in a stereotaxic frame. A scalp incision was made over the sagittal suture, and a 4 mm diameter craniotomy was performed. The facial nerve and motor branches of the trigeminal nerve were transected, and a semimicro bipolar concentric electrode (100 μm contact diameter, Rhodes Medical Instruments, Woodland Hills, CA, U.S.A.) was then stereotaxically positioned within the superior salivatory nucleus (SSN), (Paxinos & Watson, 1986; Spencer *et al.*, 1990) at coordinates previously shown to allow selective activation of the preganglionic parasympathetic innervation to the rat orbit

[1] Author for correspondence.

(Beauregard & Smith, 1994). Tarsal muscle tension was recorded as described previously (Smith *et al.*, 1983; Smith 1985; Smith *et al.*, 1987; Beauregard & Smith, 1994).

Statistics

Responses to stimulation and drugs were compared statistically by one way repeated measures ANOVA and Student Newmann-Keulls test. All values are presented as mean ± s.e.mean.

Drugs

Pharmacological agents administered through the femoral venous cannula included: physostigmine (0.1 mg kg^{-1}, i.v.), phentolamine (10.0 mg kg^{-1}, i.v.), guanethidine (25.0 mg kg^{-1}, i.v.), bethanechol (0.25 mg kg^{-1}, i.v.), atropine methyl nitrate (0.4 mg kg^{-1}, i.v.), and gallamine (10.0 mg kg^{-1} i.v.).

Results

Stimulation of the SSN alone resulted in a slight decrease in tarsal muscle tension (-113 ± 11 mg, Figure 1a and 1b), as reported previously (Smith & Beauregard, 1993; Beauregard & Smith, 1994). Administration of physostigmine in the absence of stimulation resulted in a gradual increase in muscle tension that reached its maximum (525 ± 33 mg) in approximately 1 min; this is consistent with reports that cholinesterase inhibition can increase sympathetic tone by eliciting spontaneous discharge of ganglionic neurones (Gilman *et al.*, 1990) and by activating central sympathoadrenal pathways (Kennedy *et al.*, 1984). Tension returned to baseline values over a period of 5 to 10 min. After tension had returned to baseline, parasympathetic stimulation now resulted in a large, abrupt contraction (500 ± 46 mg, $P<0.001$, Figure 1a and 1b).

To assess the role of cholinoceptors in this contractile response, the muscarinic antagonist, atropine and the nicotinic blocking agent, gallamine, were administered. Muscle contraction was unaffected by atropine (488 ± 43 mg); however, gallamine prevented the contractile response (25 ± 14 mg, $P<0.05$, Figure 2).

To determine if adrenergic sympathetic neurotransmission is required to produce muscle contraction following physostigmine, we administered the sympatholytic agent, guanethidine or the α-adrenoceptor blocking agent, phentolamine. Both guanethidine and phentolamine eliminated the contraction evoked by parasympathetic activation (-12 ± 12 mg, $P<0.05$, and 56 ± 21 mg, $P<0.05$ respectively, Figure 3).

Discussion

This study shows that AChE is important in regulating autonomic neurotransmission within smooth muscle of the rat orbit. Parasympathetic innervation of rat tarsal smooth muscle normally inhibits sympathetically mediated muscle contraction, but does not exert direct effects on the muscle (Beauregard & Smith, 1994); thus the parasympathetic innervation acts strictly to reduce sympathetic effects. However, following the administration of physostigmine parasympathetic activation elicits muscle contraction by stimulation of sympathetic nerve terminals. Therefore, the effect of parasympathetic innervation on target function can be altered fundamentally by changes in ACh degradation.

While parasympathetic activation following cholinesterase blockade results in muscle contraction, the mechanism of this excitation differs from that which occurs following sympathectomy. Contractions produced by parasympathetic activation after sympathectomy are due to activation of excitatory muscarinic receptors located on the smooth muscle, and hence, are eliminated by atropine (Smith & Beauregard, 1993). In contrast, contractions observed during the administration of physostigmine are unaffected by atropine. However, these contractions are blocked by the nicotinic antagonist, gallamine, an agent that normally does not suppress orbital parasympathetic function (Beauregard & Smith, unpublished). Therefore, different sets of cholinoceptors mediate these two atypical parasympathetic excitatory responses.

This difference is further underscored by the role of the sympathetic nervous system in these responses. In the sympathectomized preparation, the absence of sympathetic nerves precludes their participation in the production of muscle contraction. In contrast, intact sympathetic neurotransmission is essential for contraction following physostigmine administration, as this is prevented by the sympatholytic agent guanethidine. Similarly, blockade by phentolamine indicates that NA acting upon postjunctional α-adrenoceptors is responsible for contraction (Farnebo & Hamberger, 1970). Together, these results suggest that parasympathetic effects on peri-orbital smooth muscle can be converted from inhibition to excitation by 2 different mechanisms: following sympathectomy, parasympathetic activation produces muscle contraction through direct post-junctional excitation of muscarinic receptors on the smooth muscle. During pharmacological inhibition of AChE, parasympathetic stimulation evokes prejunctional excitation of

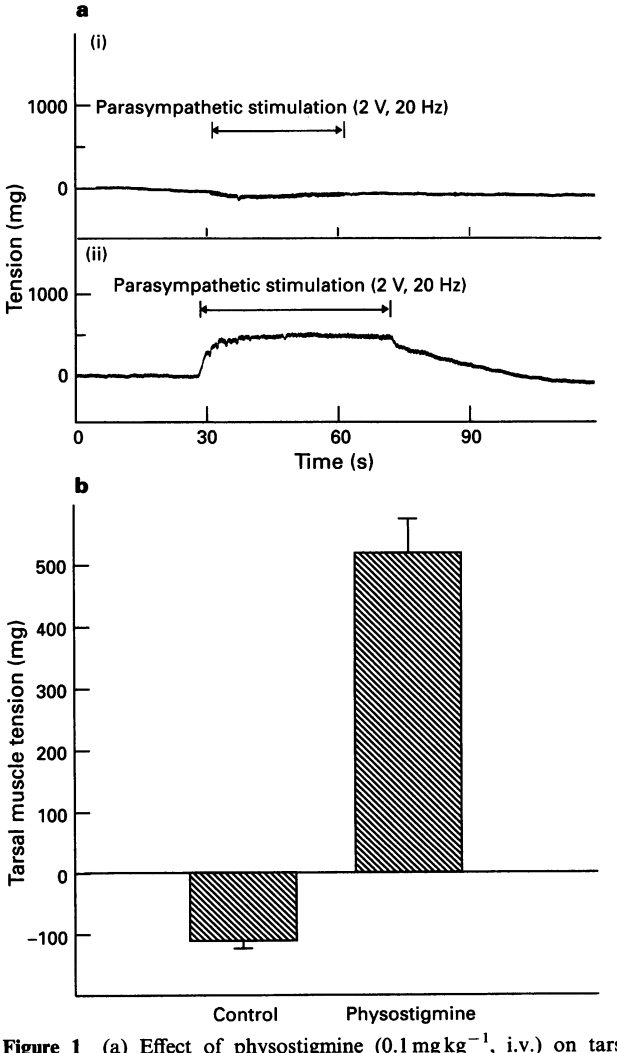

Figure 1 (a) Effect of physostigmine (0.1 mg kg^{-1}, i.v.) on tarsal muscle response to parasympathetic stimulation. Parasympathetic stimulation alone (i) caused a slight decrease in resting tarsal muscle tension. Parasympathetic stimulation following the administration of physostigmine (ii) produced a large contraction. (b) Effect of electrical stimulation of the superior salivatory parasympathetic nucleus (2V, 20 Hz) on tarsal muscle tension before (Control) and 5–10 min following the administration of physostigmine ($n=8$).

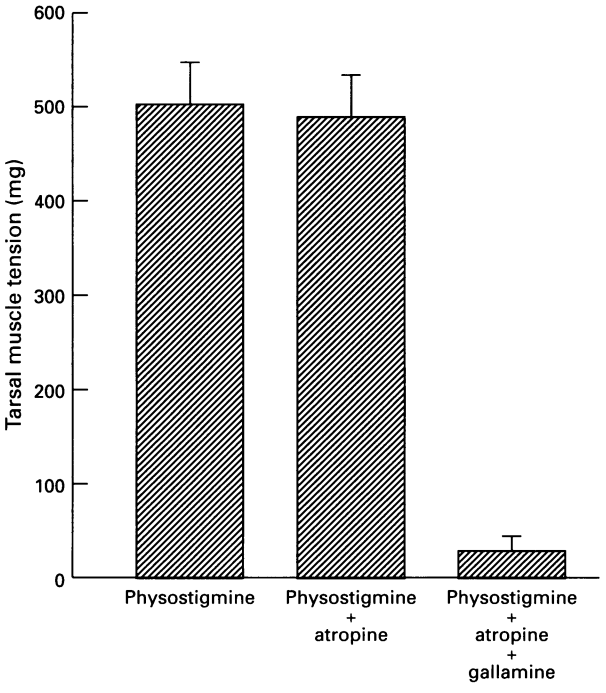

Figure 2 Effects of cholinoceptor blocking agents on tarsal muscle contractions in response to parasympathetic activation following blockade of acetylcholinesterase. The superior salivatory nucleus was stimulated in a group of rats ($n=4$) following physostigmine ($0.1\,mg\,kg^{-1}$, i.v.) administration, physostigmine plus the muscarinic blocker, atropine ($0.4\,mg\,kg^{-1}$, i.v.), and physostigmine plus atropine and gallamine ($10.0\,mg\,kg^{-1}$, i.v.), a nicotinic receptor antagonist.

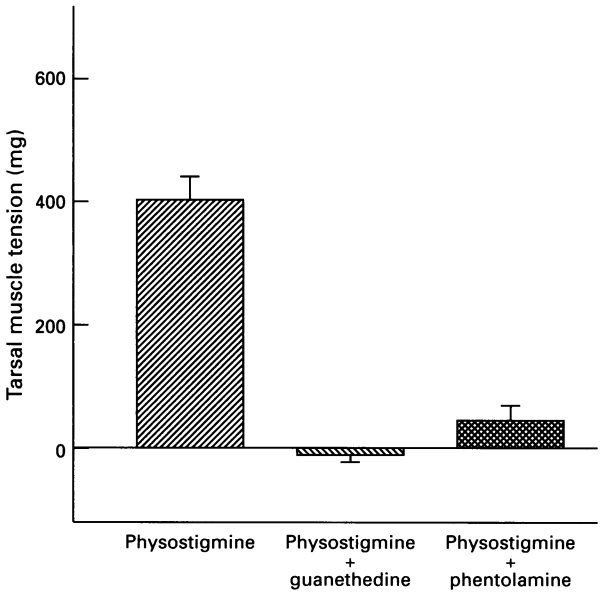

Figure 3 Effects of adrenoceptor blocking agents on tarsal muscle contractions in response to parasympathetic activation following blockade of acetylcholinesterase. The superior salivatory nucleus was stimulated in a group of rats ($n=4$) following physostigmine ($0.1\,mg\,kg^{-1}$, i.v.) administration, physostigmine plus the sympatholytic guanethidine ($25.0\,mg\,kg^{-1}$, i.v.), and physostigmine plus the α-adrenoceptor antagonist, phentolamine ($10.0\,mg\,kg^{-1}$, i.v.).

adrenergic neurones through cholinergic activation of excitatory nicotinic receptors located on the sympathetic nerve terminals.

While the mechanism of parasympathetic functional conversion following sympathectomy remains unclear, previous studies provide insight into the mechanism of conversion following AChE inhibition. Sympathetic nerve terminals possess both inhibitory muscarinic and excitatory nicotinic receptors on their axonal membranes (Haeusler *et al.*, 1968; Stjärne, 1975; Westfall, 1977; 1980; Starke, 1981; Levy, 1990; Todorov *et al.*, 1991). While the muscarinic receptors apparently have a higher affinity for acetylcholine (Haeusler *et al.*, 1968), evidence has been provided to suggest that full activation of the nicotinic receptors has more powerful effects on the nerve terminal membrane than does activation of the muscarinic receptor population (Westfall & Hunter, 1974). In rat peri-orbital smooth muscle, the action of parasympathetically released ACh is believed to be restricted under normal conditions to the inhibitory muscarinic receptors (Beauregard & Smith, 1994), thus the inhibitory action of parasympathetic stimulation on sympathetic neurotransmission. The conversion of parasympathetic nerve function following inhibition of AChE indicates that the action of AChE is important in the restriction of ACh to the muscarinic receptors; perhaps by limiting the effective diffusion radius of ACh.

This unexpected finding has important implications. Conversion of parasympathetic function from pre-junctional muscarinic inhibition to pre-junctional nicotinic excitation during the administration of cholinesterase inhibitors argues that the nerve terminal microenvironment is fundamental in determining the final transmission of target effector substances.

The potentiation of NA release from sympathetic axons produced by AChE inhibitors has clinical significance. While exaggerated parasympathetic cholinergic effects may be expected to occur in the treatment of neuromuscular disorders such as myaesthenia gravis with cholinesterase inhibitors, this study raises the possibility that potential adrenergic side effects may also occur, as over-activity of sympathetic neurones may be produced within various body systems as a consequence of AChE inhibition.

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