Role of the Sympathetic Innervation of the Pacinian Corpuscle

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ABSTRACT An investigation was made into the nature of the role played by the noradrenergic innervation of the pacinian corpuscle. Corpuscles of the cat mesentery and mesocolon were used in all experiments. Blockade of noradrenergic *beta* receptors by dichloroisoproterenol and interference with norepinephrine release by reserpine are each capable of reversibly blocking mechanoelectric transduction by the pacinian corpuscle. The monoamine oxidase inhibitors iproniazid and phenelzine are capable of protecting the transducer from the blocking effects of reserpine. It is concluded that the presence of norepinephrine, as maintained by sympathetic tonus, is required for the afferent nerve terminal of the pacinian corpuscle to be mechanosensitive.

INTRODUCTION

The ability of epinephrine and norepinephrine to lower the mechanical firing threshold of the pacinian corpuscle is well-known (Loewenstein and Altamirano-Orrego, 1956). When Santini et al. (1971) demonstrated the presence of noradrenergic fibers in close proximity to the afferent nerve terminal of the pacinian corpuscle, they raised the question of whether these fibers might play a significant role in modifying the behavior of the transducer.

Indeed, the studies in which the unmyelinated terminal of the afferent axon of the pacinian corpuscle was found to be the locus of energy transduction (Loewenstein and Rathkamp, 1958) preceded the earliest observation that the corpuscles are additionally innervated (Loewenstein et al., 1962) and, thus, never eliminated the possibility that the adrenergic fibers might themselves be the primary mechanosensitive structures with generator potential production in the afferent axon, a secondary process. The present investigation is concerned with the effect of the noradrenergic innervation in modulating or enabling transducer responsiveness and, further, addresses the question of whether the noradrenergic fibers are themselves the site of the initial energy transduction.

METHODS

Sections of cat mesentery and mesocolon containing one or more pacinian corpuscles were removed under Nembutal anesthesia. The methods of mounting the preparations, of mechanically stimulating individual pacinian corpuscles by an electrically driven piezoelectric crystal, and of recording propagated responses have been described previously (Schiff and Loewenstein, 1972).

The preparations were bathed in a Krebs solution of the following composition (mM): NaCl, 116.5; KCl, 5.9; CaCl$_2$, 2.5; MgSO$_4$, 1.18; NaH$_2$PO$_4$, 1.18; NaHCO$_3$, 25.0; D-glucose, 8.8; and CO$_2$ to pH 7.2. All drugs used were added to the bathing medium; these included dichloroisoproterenol (DCI), phentolamine (Regitine®, courtesy of Ciba Corp., Summit, N. J.), reserpine (as parenteral solution, U.S.P.), iproniazid phosphate (IPZ), and phenelzine sulfate (PLZ).

When a pacinian corpuscle is mechanically stimulated, the deformation of its capsule is in linear proportion to the driving voltage applied to the piezoelectric crystal used for stimulation. Therefore, all data on mechanical sensitivity were recorded in terms of the minimum voltage pulse, of 0.2-ms duration, which, applied to the crystal, could elicit a consistent action potential response from the pacinian corpuscle. Because of the wide variation in sensitivity which exists in normal populations of pacinian corpuscles, all data were normalized with respect to the control threshold level and were analyzed on a logarithmic scale.

RESULTS

DCI

Addition of 25 µM or greater concentration of DCI to the bathing medium brought about an increase in mechanical threshold within 15–20 min of application and caused a complete block of mechanoelectric transduction within 60 min (Fig. 1). Washing of the preparation with Krebs solution free of DCI restored mechanoresponsiveness within a similar time period.

Phentolamine

Addition of phentolamine, 100 µM, to the bathing medium produced no significant change in mechanical sensitivity over a period of 60 min (Fig. 1).

Reserpine

Addition of reserpine in concentrations of at least 25 µM to the bathing medium brought about an abrupt increase in the mechanical threshold which culminated in a complete block of transduction within 5–30 min after drug application (Fig. 2). When reserpine-treated pacinian corpuscles were washed with normal Krebs solution within 15–20 min after addition of the drug and before the blockade of transduction had become total, transducer responsiveness was generally, although not universally, restored. When preparations
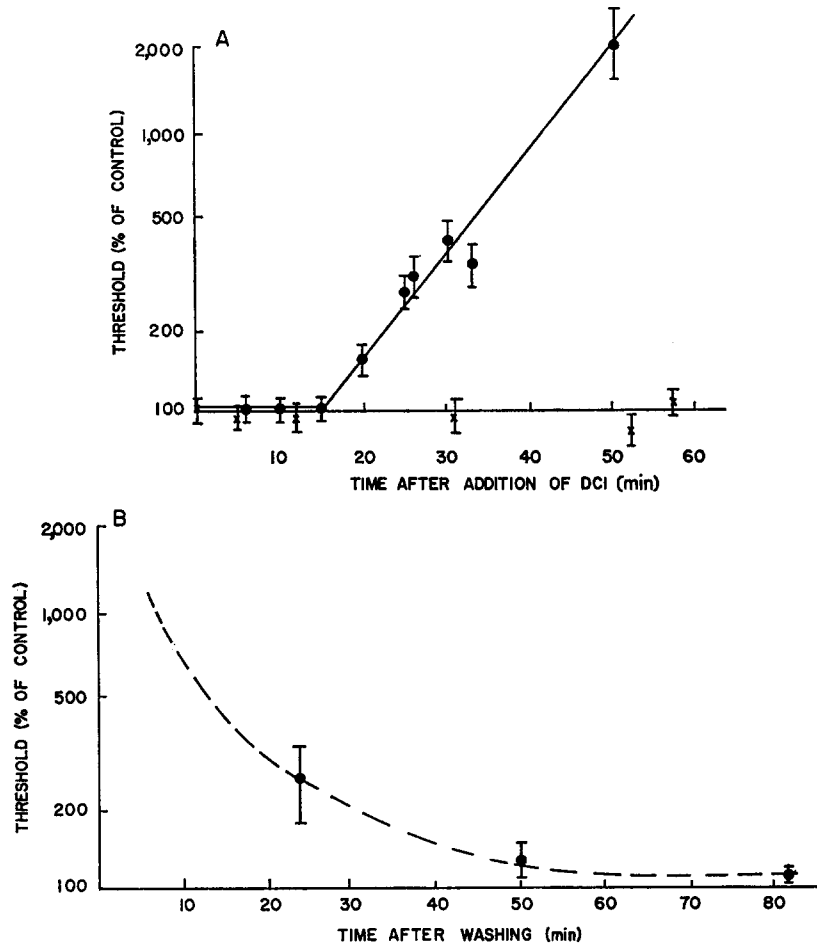
FIGURE 1. Effect of adrenergic blocking agents on mechanical threshold of pacinian corpuscle. (A) Upper curve (●), increase in mechanical threshold following addition of DCI, 25 μM. Lower curve (✕), response to addition to phentolamine, 100 μM. (B) Recovery of mechanosensitivity with washing after complete blockade by DCI, 100 μM. Each point is the mean ($\pm$ SD) of six experiments.

had been exposed to reserpine for periods in excess of 20 min, the blockade was irreversible over at least 60 min of washing.

Monoamine Oxidase Inhibitors

IPZ and PLZ, both of which are inhibitors of monoamine oxidase (MAO) (Pletscher, 1966), had no significant effect upon the mechanical threshold of pacinian corpuscles when added to the bathing medium in concentrations of 100–200 μM (Fig. 3).
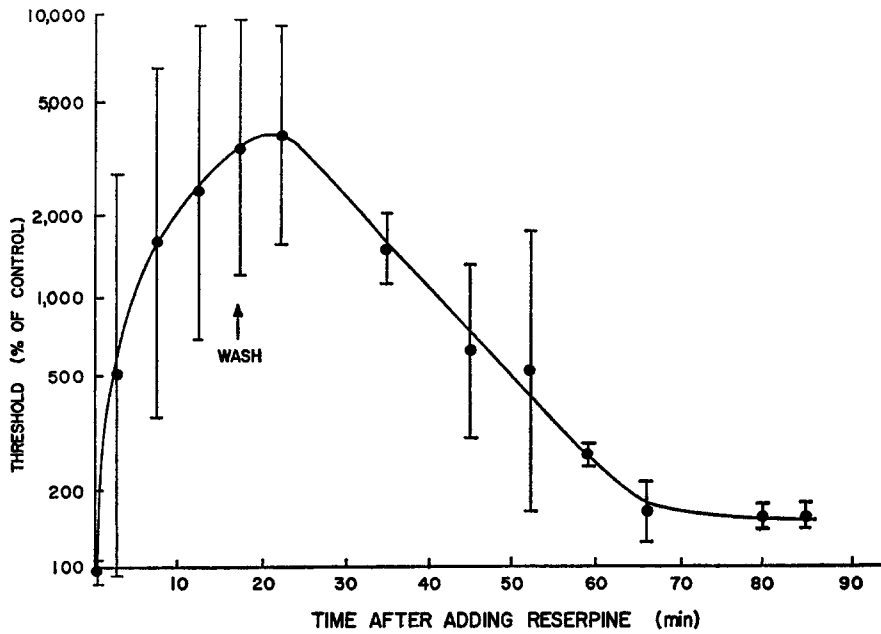
FIGURE 2.   Reversible blockade of transduction in the pacinian corpuscle by reserpine, 25 μM. Drug was washed off after 17 min of exposure. Each point is the mean (± SD) of eight experiments.
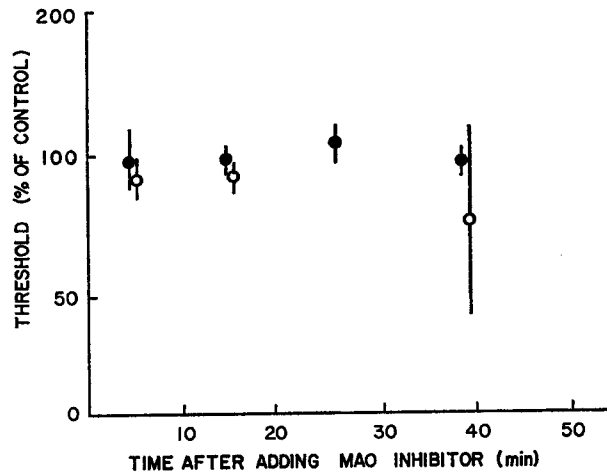

FIGURE 3.   Effect of MAO inhibitors on mechanical threshold of pacinian corpuscle. (●), iproniazid, 200 μM; (O), phenelzine, 100 μM. Each point is the mean (± SD) of five experiments.

MAO Inhibitors with Reserpine

When a pacinian corpuscle had been incubated with a MAO inhibitor for 20 min, addition of reserpine, 25 μM, in the presence of the MAO inhibitor brought about a biphasic effect: after a brief period of increased sensitivity to mechanical stimuli, a slowly deepening blockade ensued (Fig. 4). The onset of reserpine blockade in the presence of MAO inhibitor was significantly slower ($P < 0.02$) than the onset of the blockade caused by reserpine alone.
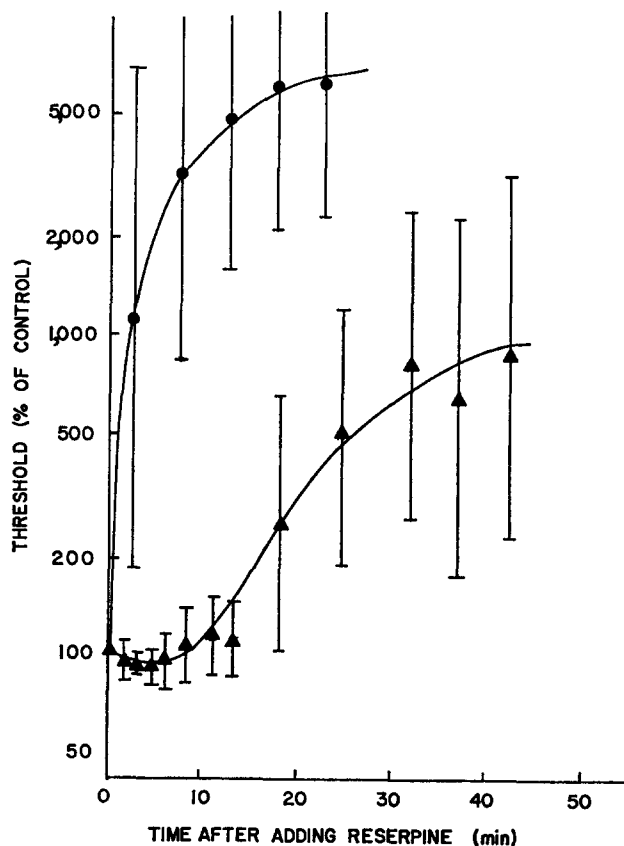

FIGURE 4. Effect of reserpine, 25 μM, on mechanical threshold of pacinian corpuscle. Upper curve (●), reserpine alone. Each point is the mean ($\pm$ SD) of 11 experiments. Lower curve (▲), reserpine in the continued presence of MAO inhibitor. Each point is the mean ($\pm$ SD) of eight experiments, four with IPZ, 200 μM, and four with PLZ, 100 μM.

DISCUSSION

The ability of either DCI, which is a *beta* adrenergic blocking agent, or reserpine, which disrupts the mechanism of norepinephrine (NE) release from sympathetic nerve endings, to interfere with mechanoelectric transduction in

the pacinian corpuscle implies the existence of a noradrenergically mediated step in the transducer mechanism, either as a part of the mechanoelectric conversion or as a parallel, enabling or gating process. That bath-applied NE reduces the mechanical threshold without inducing spontaneous spiking (Loewenstein and Altamirano-Orrego, 1956) suggests the latter.

Although DCI is generally taken to be a *beta* adrenergic blocking agent, it has recently been shown to have the ability to block *alpha* adrenergic receptors as well (Gulati et al. 1969). The inability of phentolamine, which is an *alpha* adrenergic blocking agent, to interfere with transducer function indicates that the interaction of NE within the pacinian corpuscle must be with *beta* adrenergic receptor sites.

That the blockade by reserpine is only partially reversible can be explained by reference to the effects which this drug is known to have upon peripheral noradrenergic nerve endings. Reserpine interferes with the movement of NE (Carlsson and Waldeck, 1967) and dopamine (Rutledge and Weiner, 1967) from the sympathetic axoplasm into storage vesicles, thereby allowing the available pool of transmitter to be depleted while preventing de novo NE synthesis. The storage vesicles are consequently depleted of transmitter as NE accumulates in the axoplasm where its increased concentration interferes with reuptake (Carlsson and Waldeck, 1967) and where it is subjected to degradation by mitochondrial MAO (Kuntzman et al. 1962). Thus, if the reserpine is removed early during the onset of transducer blockade, the available vesicular pool of NE is still capable of replenishment by renewed transport of NE from the cytoplasm, while, if the exposure of the pacinian corpuscle to reserpine is prolonged, degradation of the cytoplasmic NE by MAO forestalls recovery.

Transducer Models

In view of the establishment of the involvement of norepinephrine and, hence, of the sympathetic nerve fibers in the functioning of the transducer of the pacinian corpuscle, it remains to delineate the nature of the interaction between these axons and the afferent fiber during the generator process. Models can be differentiated into two general types according to the role played by NE in transduction.

NE AS MODULATOR   The mechanoelectric transducer is localized on the afferent axon of the pacinian corpuscle and, in order for transduction to occur, at least a minimum population of *beta* adrenergic receptor sites on the afferent fiber must be occupied by NE. The density of occupation determines the mechanosensitivity of the transducer.

NE AS TRANSMITTER   The conversion from mechanical stimulus to electrochemical response occurs in two stages: initially, mechanical deformations or displacements of the sympathetic fibers cause these to liberate NE; the

released NE then interacts synaptically with *beta* receptor sites on the afferent axon terminal and brings about a depolarization of this axon (the generator potential) which is analogous to an excitatory postsynaptic potential.

If the former be the case, the transducer is dependent upon the presence of NE in the core region of the pacinian corpuscle and its threshold is modulated by the level of sympathetic tonus; if the latter, depolarization of the afferent nerve terminal is in response to coordinated release of NE by the sympathetic fibers when they undergo mechanical deformation or displacement. The experiments in which reserpine was added to the bathing medium in the continued presence of MAO inhibitor were performed to distinguish between the above two hypotheses. Reserpine causes the release of NE into the region surrounding the afferent fiber while the inhibition of MAO blocks the usual major pathway for NE degradation. Thus, upon addition of reserpine, the concentration of NE in the core region of the corpuscle abruptly increases and then, by diffusion and through the action of catechol O-methyl transferase, slowly declines; the NE concentration during this period is independent of any depolarization or deformation of the sympathetic fibers as these have been depleted of their available transmitter stores.

If the first-described mechanism, wherein NE plays a modulating role in transduction, is valid, a decrease in mechanical threshold should occur and should be followed by a blockade which deepens at a rate much lower than that of the blockade caused by reserpine alone. As can be seen in Fig. 4, this is precisely what has been observed. The sensitivity plots for reserpine blockade alone and in the presence of MAO inhibitor differ by 2.5 σ.

If, on the other hand, the second mechanism which involves NE as a synaptic transmitter were valid, a burst of spikes independent of mechanical stimulation and corresponding to drug-induced transmitter release (Burn and Rand, 1958) would be expected upon the addition of reserpine in the presence of MAO inhibitor (this was not observed) while the threshold for response to mechanical deformations, which would be limited by the contents of the available vesicular pool of NE, would follow the same time-course of blockade as that produced by reserpine in a native preparation. Thus, it is apparent that mechanoelectric transduction in the pacinian corpuscle does not directly involve the sympathetic fibers, although their presence and function is necessary, but, rather, takes place entirely on the afferent nerve terminal.

CONCLUSIONS

It has been demonstrated that the sympathetic postganglionic nerve fibers which enter the core of the pacinian corpuscle, while not themselves the site of mechanoelectric conversion, play an important role in modulating the

sensitivity of the end-organ and can, at low levels of sympathetic tonus, exert a gating effect on mechanosensitivity.

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