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Transsynaptic impulse activity regulates postsynaptic density molecules in developing and adult rat superior cervical ganglion

(transsynaptic regulation/calmodulin)

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ABSTRACT **Ganglionic postsynaptic density protein (PSDp) was used to monitor the influence of transsynaptic impulse activity on synaptic structure in the developing and adult rat superior cervical sympathetic ganglion (SCG). Since transsynaptic activity is known to regulate ontogeny of postsynaptic transmitter enzymes, we initially studied the developing ganglion. Denervation in neonates prevented normal development, decreasing calmodulin binding to the ganglionic PSDp by 71% after 4 weeks. During this period, denervation elicited only a 42% decrease in total protein of the synaptic membrane fraction, suggesting that innervation regulates development of various synaptic components differentially. Effects of denervation were extremely rapid, resulting in a 44% decrease in calmodulin binding within 1 day, consistent with regulation by a signaling process such as impulse activity. The effect of impulse activity was examined more directly in adults by treatment with the agents reserpine or phenoxybenzamine, which elicit reflex increases in sympathetic transmission. Administration of reserpine resulted in a progressive 90% increase in calmodulin binding to the PSDp over 4 weeks. Phenoxybenzamine also elicited an increase, mimicking the effects of reserpine. Neither agent altered total protein of the synaptic membrane fraction, suggesting that impulse activity regulates specific synaptic components. Finally, ganglionic denervation in adults decreased PSDp binding within 12 hr, consistent with acute effects of impulse reduction. Our results suggest that transsynaptic impulse activity plays an important role in regulation of specific molecular components of the synapse.**

Although abundant evidence indicates that transsynaptic impulse activity regulates postsynaptic transmitter traits in the sympathetic system (1–5), relatively little is known about the factors that govern synaptic structure itself. Recently, however, we have found that presynaptic innervation in sympathetic ganglia influences postsynaptic molecular structure (6).

Our studies indicated that postsynaptic densities (PSDs), proteinaceous disc-shaped structures attached to the postsynaptic membrane at chemical synapses (7–10), which may play a critical role in synaptic function (11–13), are detectable in the rat sympathetic superior cervical ganglion (SCG); the PSDs exhibit morphological and biochemical characteristics comparable to those of brain (6). The presumptive ganglionic PSD protein (PSDp) was phosphorylated in the presence of Ca^{2+} and calmodulin, bound ^{125}I-labeled calmodulin, and exhibited an apparent molecular mass of 51 kDa, all characteristic of the brain major PSDp. In brief, the peripheral as well as the central PSDp appears to be an autophosphorylatable Ca^{2+}/calmodulin-dependent protein kinase.

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During postnatal development, calmodulin binding to the ganglionic PSDp increased 411-fold from birth to 60 days. Moreover, denervation of the ganglion in adult rats elicited an 85% decrease in ganglionic PSDp calmodulin binding without a change in synaptic membrane protein 2 weeks postoperatively. Our observations suggested that presynaptic innervation in some manner regulated the ganglionic PSDp.

In the present study, we attempted to define mechanisms through which presynaptic innervation regulates the PSDp in the rat SCG. We used a pharmacological approach to determine whether transsynaptic impulse activity itself regulates the PSDp. In fact, reflex increases in sympathetic activity, evoked by reserpine or phenoxybenzamine, drugs that interfere with sympathetic physiological function through different mechanisms (14, 15), increased calmodulin binding to the ganglionic PSDp. Conversely, surgical denervation of the SCG in adults elicited a rapid decrease in PSDp binding within 12 hr. Finally, denervation of the SCG in neonates prevented the normal development of the PSDp. In aggregate, our observations suggest that transsynaptic impulse activity itself regulates synaptic molecular structure in the sympathetic ganglion.

MATERIALS AND METHODS

Materials. $Na^{125}I$ was obtained from Amersham. Methyl-4-azidobenzimidate was purchased from Pierce. Calmodulin was a product of Boehringer Mannheim. The other chemicals are of highest grades from commercial sources.

Experimental Animals and Dissection of SCG. Sprague–Dawley rats were used in the present study. The animals were maintained in a temperature-controlled room as described (6). Animals were killed by exposure to CO_2 vapor and SCGs were removed as reported (6). The pooled SCGs were stored at −80°C and used within 1 day.

Ganglion Denervation (Decentralization). Neonatal rats were anesthetized on ice for 5 min, and denervation was performed at room temperature as described (6). The wounds were sealed with flexible colloidin and the animals were allowed to grow for various periods of time. Adult rats were anesthetized with fluothane and denervation was carried out as described above.

Subcellular Fractionation. Synaptosomal and synaptic membrane (SM) fractions were prepared from pooled frozen SCGs according to published procedures (16).

Binding of Calmodulin to the Ganglionic PSDp. Binding of ^{125}I-labeled azidocalmodulin to the PSDp in the ganglionic SM fractions and quantification of bound labeled calmodulin were performed as described (6). Previous work has indicated that the protein is exclusively localized in the PSD itself in the SM fractions (17).

Pharmacological Procedures. Rats (3 months old) were injected subcutaneously with saline (control) or reserpine (2

Abbreviations: PSD, postsynaptic density; PSDp, PSD protein; SCG, superior cervical ganglion; SM, synaptic membrane.

mg per kg of body weight) every other day for various periods of time. The SCGs were removed for subcellular fractionation at various times after the treatment. Alternatively, adult rats were injected with saline or phenoxybenzamine (25 mg per kg of body weight) twice a day for a period of 7 and 21 days before the ganglia were isolated for subcellular fractionation.

RESULTS

Effects of Denervation on Development of Ganglionic Synapses. Transsynaptic impulse activity is known to be necessary for normal development of transmitter enzymes in postsynaptic neurons of the rat SCG (4, 5). To begin to determine whether impulse activity also potentially regulates synaptic structure, ganglia were denervated in neonatal rats. The SCG was decentralized unilaterally and the contralateral intact ganglion in each animal served as a control. At various postoperative times ganglia were removed, and calmodulin binding to the PSDp was assayed. In fact, decentralization prevented the normal ontogeny of calmodulin binding to the ganglionic PSDp (Fig. 1 *Upper*): binding was 25% of control 3 days postoperatively (*Lower*).

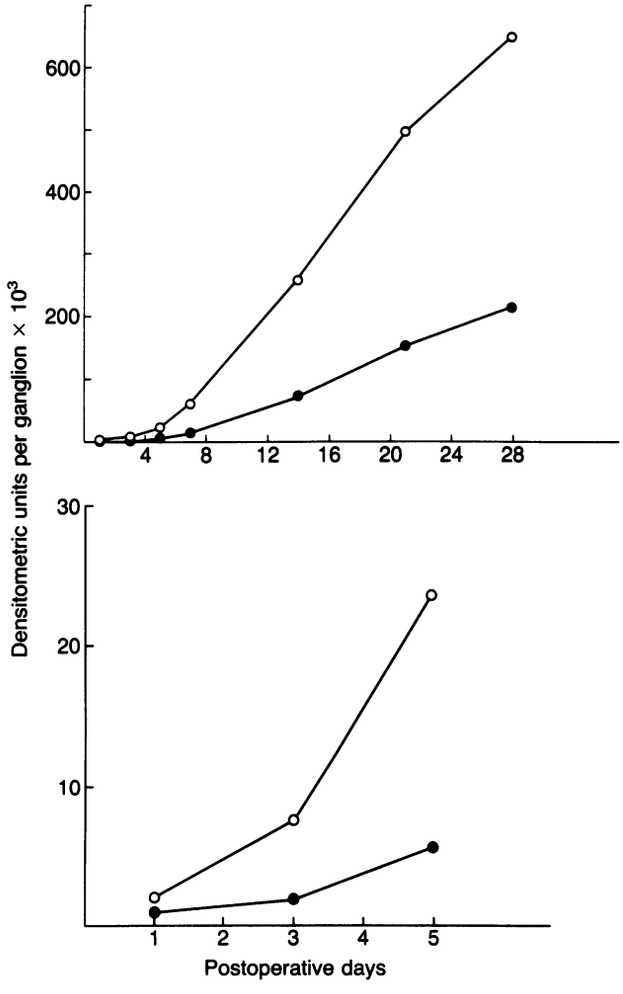

FIG. 1. Effect of denervation on calmodulin binding to the ganglionic PSDp of developing rats. Ganglia in neonates (day 1) were unilaterally decentralized and the animals were allowed to grow for various periods of time. The SCGs from both denervated and contralateral (control) sites were removed for biochemical assay of the PSDp. (*Upper*) Results obtained between days 1 and 28. (*Lower*) Expanded scale between days 1 and 5, indicating rapidity of the changes. ○, Control; ●, denervated.

To assess the effect of decentralization on the synapse as a whole, to ascertain the specificity of the effect of surgery on the PSDp, and to define growth of the overall synapse during development, total protein of the SM fraction was measured (Fig. 2). In control ganglia, protein of the SM fraction increased from 0.7 μg per ganglion on day 1 of life to 3.1 μg per ganglion by day 28. In contrast, SM protein was 1.8 μg per ganglion after decentralization on day 28. Consequently, denervation decreased total synaptic protein to ≈58% but decreased the ganglionic PSDp to 29%.

Effects of Pharmacologically Induced Increases in Impulse Activity on the Synapse. To begin determining whether transsynaptic activity itself regulates molecular components of the synapse, impulse activity in the sympathetic system was increased by administering reserpine, a drug that causes a reflex increase in sympathetic outflow by depleting catecholamine stores (14). In fact, chronic treatment of adults with reserpine elicited a progressive increase in PSDp calmodulin binding (Fig. 3). PSDp binding increased ≈2-fold after 28 days of treatment, the longest time tested. In contrast, total protein of the synaptosomal or SM fraction was not significantly altered by reserpine treatment (data not shown), suggesting that transsynaptic activity specifically regulates the PSDp. $NaDodSO_4$/PAGE analysis confirmed these observations, indicating that treatment did not alter the pattern of protein of the SM fraction (data not shown).

To ascertain whether the effect of reserpine was actually mediated by increased transsynaptic activity, a wholly different agent was used. Phenoxybenzamine, structurally and pharmacologically different from reserpine, increases sympathetic impulse activity by blocking α-adrenergic receptors in target organs (15). Treatment with the α-adrenergic receptor antagonist for 7 or 21 days increased PSDp binding by 26% and 47%, respectively (Fig. 4), reproducing the effects with reserpine and suggesting that increased impulse activity itself regulates the ganglionic PSDp. Phenoxybenzamine elicited no change in either synaptosomal or SM contents, reproducing observations made with reserpine (data not shown).

Acute Effects of Surgical Denervation in the Adult. Previously, we reported that presynaptic denervation of the SCG in adults resulted in a 74% decrease in calmodulin binding to the PSDp within 1 week, the earliest time tested (6). However, if impulse activity is the critical presynaptic factor regulating the PSDp, effects may be evident far more rapidly. To examine this possibility, ganglia were unilaterally decen-

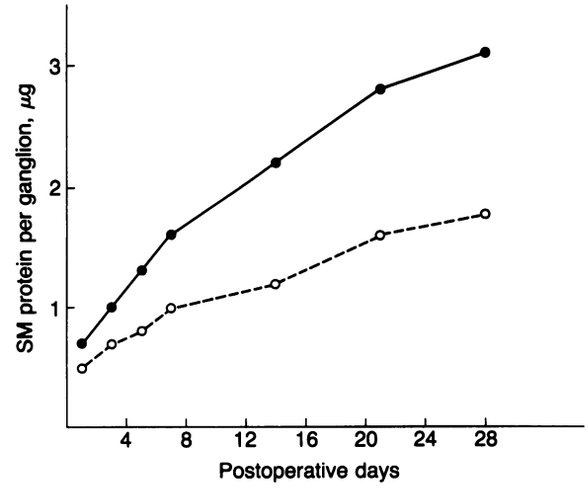

FIG. 2. Developmental increase in total protein of SM fraction in control and denervated SCGs of rat. Neonatal rats were subjected to unilateral ganglion denervation and ganglia were removed at various postoperative times. SM proteins were prepared from the SCGs. ●, Control; ○, denervated.

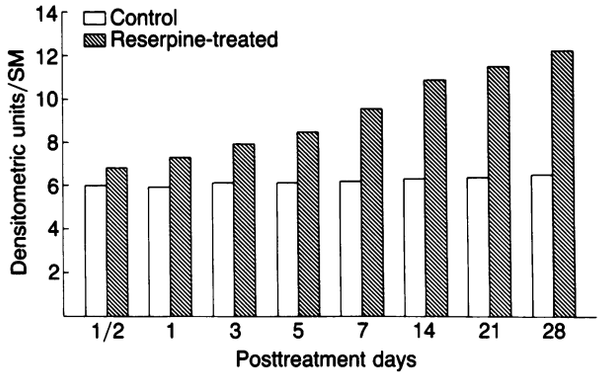

FIG. 3. Effect of reserpine treatment on binding of calmodulin to the ganglionic PSDp. Rats (3 months old) were injected with saline (control) or reserpine subcutaneously at a dose of 2 mg per kg of body weight every other day for various periods. The PSDp from the drug-treated and control SCGs were assayed by calmodulin binding.

tralized in adults and the PSDp was assessed at various times postoperatively. As early as 12 hr after denervation, there was a 40% decrease in calmodulin binding to the PSDp (Fig. 5). By day 5, binding had decreased to 34%. Total protein of the SM fraction was used as a measure of the synapse as a whole, and this was unchanged after decentralization (data not shown), as indicated (6).

DISCUSSION

The present studies were designed to ascertain whether regulation of the postsynaptic density by presynaptic innervation in the SCG was potentially mediated by impulse activity. Several lines of evidence derived from the current experiments suggest that transsynaptic transmission does, in fact, regulate the PSDp in the ganglion.

In initial experiments, we examined the effect of denervation on the development of the PSDp, since extensive evidence indicates that transsynaptic impulse activity regulates the development of multiple postsynaptic neuronal

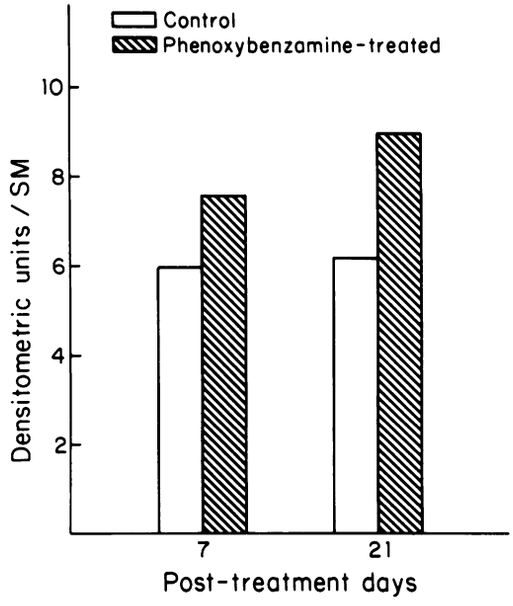

FIG. 4. Effect of phenoxybenzamine treatment on binding of calmodulin to the ganglionic PSDp of adult rat. Experimental conditions were similar to those described in Fig. 3, except that phenoxybenzamine (25 mg per kg of body weight, twice a day) was used.

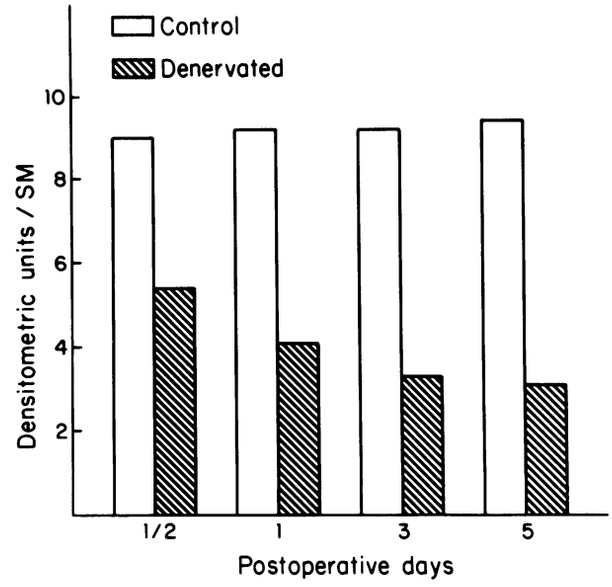

FIG. 5. Effect of denervation on binding of calmodulin to the adult ganglionic PSDp of rat. Rats (3 months old) were subjected to unilateral SCG decentralization and allowed to survive for various periods of time. The PSDp in SCGs were isolated from denervated and control ganglia and were assayed for calmodulin binding.

biochemical characters (4, 5). Indeed, in the present study, decentralization prevented the normal development of the ganglionic PSDp, providing circumstantial evidence that impulse activity itself influences the development of at least one integral synaptic protein. It is particularly noteworthy that the effects of denervation on the PSDp were extremely rapid, resulting in a reduction to 56% within 24 hr of denervation, further supporting the contention that interruption of a rapid signaling process, such as impulse activity, might potentially have been involved.

Denervation of the SCG in the neonate appeared to exert differential longer term effects on different components of the synapse. Four weeks postoperatively, the PSDp was reduced to 29%, while total SM protein was decreased only to 58%. Our observations raise the possibility that impulse activity differentially regulates individual components of the synapse.

The foregoing experiments, performed in neonates, provided only indirect evidence concerning the role of impulse activity in synaptic regulation. To approach this issue more directly, pharmacological agents were used to elicit a reflex increase in sympathetic impulse activity in adults. Reserpine and phenoxybenzamine, structurally dissimilar agents that increase sympathetic activity through entirely different mechanisms (see *Results*), both increased the ganglionic PSDp. Our observations thus suggest that impulse activity *per se* regulates the PSDp.

We studied acute effects of denervation in the adult, as in the neonate, to determine whether the influence of innervation was ongoing and rapid, potentially implicating a mechanism such as impulse transmission. Denervation resulted in extremely rapid change in the PSDp in the adult, as in the neonate. Indeed, within 12 hr of decentralization, there was a 40% decrease in the binding of calmodulin to the PSDp. Once again, denervation appeared to selectively alter the PSDp, since total protein of the synaptic membrane fraction was unchanged. The selectivity of effects is in agreement with previous work indicating that denervation of the SCG reduced total synapse number without destroying PSDs (18, 19). We tentatively conclude that impulse activity itself may critically regulate the PSDp.

The alteration of calmodulin binding to the PSDp by the state of innervation and by impulse activity itself may be attributable to several different molecular mechanisms. For example, and most simply, decreased binding may reflect a true decrease in the number of PSDp molecules. Alternatively, changes in the conformation of the PSDp or changes in the binding of endogenous calmodulin may have altered the binding of exogenous calmodulin. Regardless of underlying molecular mechanisms, however, our observations suggest that impulse activity regulates specific components of the synapse selectively in sympathetic neurons of the adult and neonatal rats.

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