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SMALL INTENSELY FLUORESCENT (SIF) CELLS AND NERVOUS TRANSMISSION IN SYMPATHETIC GANGLIA[1]

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INTRODUCTION

Several early observations suggested that sympathetic ganglia are not merely simple relay stations. Chromaffin cells were observed in some sympathetic ganglia (1). Injections of catecholamines (2) or ganglion perfusates collected during preganglionic stimulation (3) were found to modify the postganglionic action potentials obtained in response to fixed preganglionic stimuli. Both reserpine-sensitive and reserpine-resistant catecholamine stores were observed in sympathetic ganglia (4). In a paper that has since stimulated many studies, Eccles & Libet (5) observed that on curarized superior cervical ganglion of the rabbit the positive wave that follows upon preganglionic stimulation the primary negative synaptic potential was strongly depressed by atropine or dibenamine.

[1]The content of this review is condensed from a book in preparation by Olavi Eränkö and Liisa Eränkö: *Small Intensely Fluorescent (SIF) Cells,* to be published by Raven Press, New York.

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INTRAGANGLIONIC CATECHOLAMINE STORES

With formaldehyde-induced fluorescence catecholamines were histochemically demonstrated in the superior cervical ganglion of the rat (6). It was proposed that there were two catecholamine pools in the nerve cells, a diffuse pool and a granular one. In addition to nerve cells, small cells were observed in the ganglion which exhibited an extremely bright fluorescence (6). These cells were found to exhibit a negative chromaffin reaction, although they were shown by electron microscopy to contain small osmiophilic granules like the adrenal medullary chromaffin cells and to be close to blood vessels; it was proposed that these cells represent a new type of catecholamine-storing cell, possibly with an endocrine function (7). They were called *small intensely fluorescent (SIF) cells,* and they were observed to be present in the ganglion also after postganglionic nerve division (8). SIF cells have been demonstrated in many ganglia of several species (9–16) by fluorescence microscopic techniques.

The presence of numerous brightly fluorescent terminals around adrenergic ganglion cell bodies has been reported by a number of authors (13, 14, 17, 18). These terminals persist after preganglionic (13, 18) and postganglionic (13) nerve division. They were thought to originate from adrenergic interneurons inside the ganglion and to be responsible for adrenergic inhibition of ganglionic transmission, rather than the SIF cells (13).

Clusters of small granular vesicles 50 nm in diameter have been found in peripheral areas of sympathetic nerve cell bodies in sites corresponding to the fluorescence microscopic granules (19–23), while mainly endoplasmic reticulum has been seen in the central areas of even cytoplasmic fluorescence (23). Between the cell bodies are many dendrites with wider portions containing numerous small granular vesicles, often in close proximity to each other and near nerve cell bodies (21, 22, 24, 25). The apparent fluorescent terminals seen around adrenergic nerve cell bodies (10, 18, 26) may have been in some instances either adrenergic dendrites near, or clusters of granular vesicles inside, adrenergic nerve cell perikarya (22).

SIF cells contain numerous osmiophilic granules (7), and in the superior cervical ganglion of the rat the size of the granular vesicles is about 100 nm (20, 27–31). The same is true for the same ganglion of the guinea pig (35). This is twice the diameter of the granular vesicles in the nerve cells (see above) but only about one half the diameter of the granular vesicles in the adrenal medullary cells (32). The difference in the size of the granules probably explains why the SIF cells of the superior cervical ganglion do not exhibit a positive chromaffin reaction while the adrenal medullary cells do (29, 33). SIF cells in some ganglia, notably prevertebral abdominal and pelvic ganglia, have been shown to contain not only 100 nm granular vesicles but others whose diameter approaches 300 nm, i.e. that of the adrenal medullary vesicles: inferior mesenteric ganglia of the rat (22), the rabbit (34) and the guinea pig (35); paracervical ganglion of the rat (36, 37); the hypogastric ganglia of the guinea pig (38) and the rat (39). In some of these ganglia, indeed, cells exhibiting a positive chromaffin reaction have been found (40–44). In the inferior mesenteric ganglion of the guinea pig some SIF cells contain apparently noradrenaline-contain-

ing granules, others adrenaline-containing granules, and a third SIF cell type has both kinds of granules (35).

SYNAPTIC AND OTHER CONTACTS

Principal sympathetic nerve cells receive synapses containing empty vesicles (19, 45–47). Most of these are probably preganglionic and cholinergic but preganglionic denervation does not abolish all such synapses in the superior cervical ganglia of the rat, mouse, and guinea pig (20, 48–50). Numerous close contacts between the dendrites of the principal neurons may well serve transfer of information (45–47).

Abundant data are available on synaptic contacts to and from SIF cells, whose fine structural features are illustrated in Figure 1. In the superior cervical ganglion of the rat the SIF cells have been seen to be innervated by afferent synapses with numerous small "empty" vesicles (20, 22, 25, 27, 30, 51–56). These synapses have been seen to degenerate after division of the preganglionic nerve (30, 54, 57).

Efferent synapses in which SIF cells or their processes are the presynaptic element, as indicated by clustering of large granular vesicles, and the dendrites or perikarya of adrenergic nerve cells are the postsynaptic element have been reported in the superior cervical ganglion of the rat (25, 27–30, 52, 54–56). Such SIF cells can be expected to act as interneurons.

Afferent synapses containing empty synaptic vesicles have also been reported on SIF cells in all other ganglia as yet studied. Efferent synapses from SIF cells to principal neurons have also been found in most ganglia, including the major pelvic ganglion of the rat (39) and the paracervical ganglia of the rat (36–38, 58, 59) and the mouse (60). However, efferent synaptic contacts to nerve cells have not been found in the inferior mesenteric ganglion of the cat (47) or the guinea pig (35), or in the hypogastric ganglion (38), or the superior cervical ganglion of the guinea pig (35). Furthermore, in the superior cervical ganglion of the cow, cat, monkey, and rabbit some SIF cells have both afferent and efferent synaptic contacts, while other SIF cells have only afferent but no efferent synapses (61).

Neighboring SIF cells are often in a close contact and linked to each other with attachment plaques (20, 29, 55, 56). The satellite cell sheath around the SIF cells is often lacking when the SIF cells are near blood vessels, which are often fenestrated (20, 25, 28, 29, 51, 55).

The close relations of the SIF cells to blood vessels have been taken to suggest that they have an endocrine function (7, 20, 28, 29) possibly acting locally on the nerve cells of the ganglion through a portal circulation (27). That SIF cells may act as interneurons through their efferent synapses does not necessarily exclude the possibility that they also secrete materials by exocytosis to the intercellular space through which several nearby nerve cells can be affected (30). The presence of dense patches at the inner face of the SIF cell membrane suggests exocytosis, and catecholamines thus secreted may exert their effects even at a certain distance because amine uptake by the SIF cells has been reported to be low as compared to uptake by principal neurons (56).

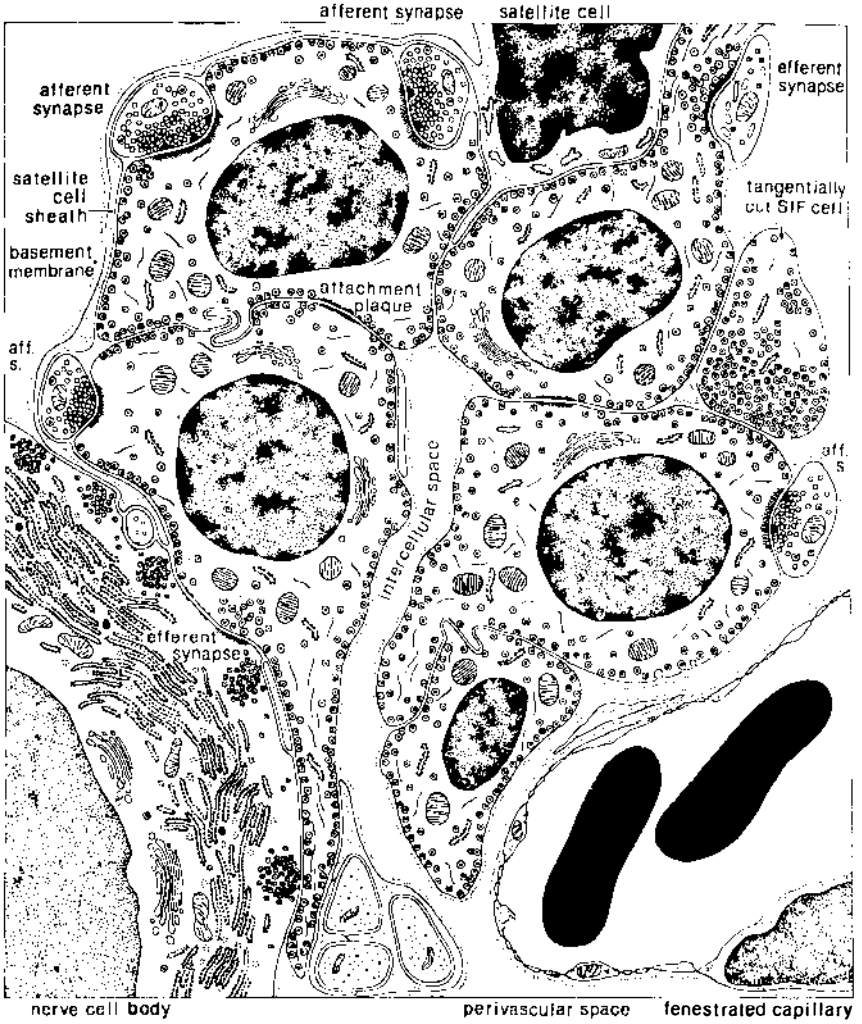

Figure 1 Schematic drawing illustrating fine structural features of six SIF cell profiles, a nerve cell body, a fenestrated capillary, and a satellite cell. Note direct apposition of neighboring SIF cell membranes and of SIF cell and nerve cell membranes, and partial covering of SIF cells by sheaths of satellite cell cytoplasm or, towards intercellular and perivascular spaces, by basement membrane. The small granular vesicles of the nerve cell are in clusters near the outer cell membrane while the large granular vesicles of the SIF cells are more evenly distributed in the periphery of the cell, presumably releasing their contents by exocytosis into the extracellular spaces. Of the four afferent synapses on the SIF cells, one (*upper left*) is opposite to a cluster of large granular vesicles, as a suggestion of a reciprocal synapse. There is an efferent somatosomatic synapse between a SIF cell and the nerve cell body and another efferent synapse from a thick SIF cell process to a nerve cell dendrite (*upper right*). Art work by Trudy Nicholson. From O. Eränkö and L. Eränkö: *Small Intensely Fluorescent (SIF) Cells,* Raven Press, New York.

Closeness to blood vessels can also be connected with chemoreceptor function (25, 36, 62), and clusters of SIF cells that are in close relation to each other, to sympathetic nerve cells, and to blood vessels may form small chemoreceptor organs resembling the carotid body (62).

Matthews & Raisman (29) observed that on occasion granular vesicles can be found clustered close to the membrane underlying an afferent synapse, raising the question whether the synapse might not be reciprocal. Yokota (55) thought that dendrites may sometimes form a reciprocal synapse with the SIF cells "providing a basis for a mechanism of disinhibition on impulse transmission through the ganglion." Taxi & Mikulajova (56) pointed out that numerous attachment plaques are present between SIF cells and profiles with empty vesicles that can be interpreted as preganglionic terminals, in the same way as in the carotid body, in which the fibers are sensory, and prompted further investigation of the sensory nature of such endings. It has recently been observed that some synapses on the SIF cells of the superior cervical ganglion of the rat degenerate after division of the sensory glossopharyngeal nerve; in some of such synapses the polarity of empty vesicles in the terminal and that of the large granular vesicles in the SIF cell opposite to it suggest that it is a reciprocal synapse (M. Grillo, to be published). Such morphologically reciprocal, functionally sensory synapses have been described on glomus cells of the carotid body (63), whose fine structure much resembles that of the SIF cells.

Also fluorescence microscopy of cholinergic parasympathetic cardiac ganglia of the rat, cat, guinea pig, and mouse has demonstrated numerous SIF cells, which apparently receive afferent synapses from the parasympathetic postganglionic axons (15, 64–68). SIF cells with 100 nm granular vesicles observed in the interatrial septum of the guinea pig heart had on them synapses with 55 nm translucent vesicles and some 95 nm dense core vesicles, which were considered afferent to the SIF cell (68); however, the published photomicrographs can also be interpreted as showing reciprocal synapses. Synapses between cholinergic terminals and SIF cells which were in serial sections seen to be reciprocal were demonstrated in the cardiac ganglia of the rat (69) and the turtle (70). Parasympathetic synapses from cholinergic nerve fibers were observed on SIF cells of the cardiac parasympathetic ganglion of the mudpuppy, while the soma or processes of the SIF cells were presynaptic to the nerve cells (71). SIF cells have also been found in the nodose ganglion of the rat (72).

DIFFERENT CATECHOLAMINES IN SIF CELLS

It has been shown by microspectrofluorometry that the SIF cells probably contain primary catecholamines (73). Making use of differential changes induced by hydrochloric acid in the fluorescence spectra of dopamine and noradrenaline fluorophores (74), it was reported that the SIF cells of the superior cervical ganglion of the cat, pig, and rat contain dopamine (75) or (rat) noradrenaline (62). Spectral characteristics typical of dopamine have also been found in the SIF cells of the superior cervical ganglion of the rabbit (76) and the cow (77).

The trihydroxyindole fluorescence method used for the same purpose also suggested that in the rat the SIF cells of the superior cervical ganglion contain dopa-

mine, while those of the paracervical ganglion contain noradrenaline (59). A modified dichromate reaction suggestive of noradrenaline has been found to be positive in the hypogastric ganglion of the rat (39).

Mass fragmentographic analysis of sections of the superior cervical ganglion of the rat whose alternate sections were shown to be free of SIF cells using formaldehyde-induced fluorescence indicated that the dopamine content was 23% of the noradrenaline content, while in the whole ganglia, which also contained the SIF cells, the dopamine content was much higher, 37% of the noradrenaline content, indicating that SIF cells contain much dopamine (78, 79). It has been estimated that 40% of the dopamine stores of the ganglion is in the SIF cells (80). Evidence has also been given for the presence of some adrenaline in the SIF cells of the superior cervical ganglion of the rat (81).

In the superior cervical ganglion of the rat, the SIF cells have been reported to contain immunohistochemically demonstrable dopa decarboxylase (82) and tyrosine hydroxylase (83) but not dopamine β-hydroxylase, although this enzyme was found in the cytoplasm of the sympathetic nerve cells (59, 82, 83); thus, in this ganglion, the SIF cells seem to be able to make dopamine but not noradrenaline. However, dopamine β-hydroxylase, but not phenylethanolamine N-methyltransferase, has been immunohistochemicallly demonstrated in the superior cervical ganglion of the guinea pig (35) and in some cells of the paracervical ganglion of the rat (59), indicating that these SIF cells can make and probably store noradrenaline. Other SIF cells in the paracervical ganglion of the rat exhibited catecholamine fluorescence but did not contain dopamine β-hydroxylase; they were interpreted to contain dopamine (59). All SIF cells of the inferior mesenteric ganglion of the guinea pig were found to contain both tyrosine hydroxylase and dopamine β-hydroxylase, and thus presumably noradrenaline, but only some of them contained phenylethanolamine N-methyltransferase, which is necessary for adrenaline synthesis (35). The above observations show that the catecholamine content of individual SIF cells can vary in a single ganglion, between different ganglia of each species, and between analogous ganglia of different species.

EFFECTS OF DRUGS ON SIF CELLS

Doses of reserpine that readily deplete the catecholamine fluorescence and the small granular vesicles from the sympathetic nerve cell perikarya and nerve fibers have been shown to cause little change in the intensity of the catecholamine fluorescence or in the electron density or number of the large granular vesicles in the SIF cells, observations that suggest that the SIF cells have a slow turnover of catecholamines (15, 22, 73, 84). In the superior cervical ganglion of the rat, doses of reserpine causing catecholamine depletion from principal neurons but not from SIF cells also cause a loss of most noradrenaline while the content of dopamine (85) or adrenaline (81) in the ganglion is little affected or unchanged; this supports the view that the SIF cells of this ganglion contain dopamine and adrenaline (80). Large doses of reserpine cause a loss of catecholamine also from SIF cells (73, 84). Principal neurons but not SIF cells are depleted with suitably selected doses of metaraminol (73) or α-methylmetatyrosine (22).

Inhibition of tyrosine hydroxylase with α-methylparatyrosine first caused a rapid loss of granular fluorescence and the small granular vesicles from the nerve cells while diffuse nerve cell fluorescence was still visible; then, all nerve cell fluorescence disappeared, and SIF cells were hardly affected; then electron density decreased in the large granular vesicles of the SIF cells but the fluorescence of these cells still changed very little (22). These observations reflect the much slower turnover rate of the SIF cells, and the preferential depletion of catecholamines from the granular, rather than extragranular pool.

Inhibition of β-hydroxylation of dopamine into noradrenaline by injections of diethyldithiocarbamate results in a loss of most noradrenaline from the superior cervical ganglion of the rat without change in dopamine concentration, presumably since dopamine is located in the SIF cells as a product, rather than as a noradrenaline precursor in the nerve cells (85).

In newborn rats, almost complete loss of sympathetic nerve cells can be caused by daily injections of 6-hydroxydopamine for a week, but the total number of SIF cells in the ganglion remains unchanged (86). Guanethidine injections also cause irreversible death of sympathetic ganglion cells in newborn rats, but the number of SIF cells in the ganglion increases three- to fivefold (87), the size of each SIF cell cluster increasing. At the same time the noradrenaline concentration decreases significantly, while the concentration of dopamine, presumably located in the SIF cells, remains unchanged (79).

In adult rats, 6-hydroxydopamine causes vacuolization of SIF cells but no changes in the nerve cells (88), while parachlorophenylalanine administration results in reversible degranulation of the SIF cells (89).

Glucocorticoids have no effect on adult ganglia but cause in newborn animals a great increase in the number of SIF cells (90), in the phenylethanolamine N-methyltransferase activity (91), and in the adrenaline content (81). While only tyrosine hydroxylase is immunohistochemically found in the SIF cells of normal one-week-old rats, daily administration of hydrocortisone for a week to newborn rats causes an increase in the tyrosine hydroxylase activity and the appearance of dopamine β-hydroxylase, phenylethanolamine N-methyltransferase, and membrane protein in the numerous newly formed SIF cells (O. Eränkö, V. Pickel, M. Härkönen, L. Eränkö, T. Joh, and D. Reis, to be published). In organ cultures of sympathetic ganglia, addition of hydrocortisone in the culture medium causes an increase in the number of SIF cells, in the intensity of their fluorescence, and in the number of large granular vesicles in them (92).

MODULATION OF GANGLIONIC TRANSMISSION BY SIF CELLS

In the rabbit superior cervical ganglion, preganglionic stimulation elicits three types of responses: a fast negative excitatory postsynaptic potential (fast EPSP), a slow positive inhibitory postsynaptic potential (s-IPSP), and a slow negative excitatory postsynaptic potential (s-EPSP) (93–95). The fast EPSP is nicotinic, being abolished by curare, while s-IPSP and s-EPSP can be abolished either by atropine, these potentials being mediated by muscarinic synapses, or by the α-blocker dibenamine,

which also abolishes the effect of adrenaline on ganglionic potentials. It was proposed that the s-IPSP is due to stimulation by acetylcholine of muscarinic receptors on postulated chromaffin cells and subsequent adrenaline release from them, which encounters s-IPSP receptor sites on ganglion cells and causes hyperpolarization of the ganglion cell membrane, thus producing the s-IPSP potential (5). SIF cells have subsequently been proposed as cells responsible for the inhibitory action and s-IPSP (27).

Because lowering the Ca/Mg ratio just sufficiently to abolish orthodromic synaptic transmission also selectively abolished the hyperpolarizing response but not the depolarizing response to muscarinic agonists, both in the rabbit superior cervical ganglion (94) and the frog paravertebral ganglion (96), it was concluded that synaptic release of a noncholinergic transmitter was necessary to elicit s-IPSP upon stimulation of cholinergic preganglionic fibers (95). Microspectrofluorometric evidence of dopamine in the SIF cells and fluorescent endings around nerve cell bodies of rabbit superior cervical ganglion, concomitant loss of histochemically demonstrable dopamine and of the s-IPSP response, induced by conditioning preganglionic stimulation in the presence of α-methylparatyrosine to inhibit dopamine synthesis, and concomitant recovery of SIF cell dopamine and the s-IPSP response after further incubation with dopamine (76), strongly support the view that dopamine released from SIF cell terminals is responsible for s-IPSP [Figure 2 (95)]. The s-IPSP reduced upon conditioning preganglionic stimulation cannot be restored by exposure to noradrenaline or adrenaline (97). The s-IPSP response is enhanced by blockers of catechol *o*-methyltransferase; it is depressed by α-adrenergic blockers but remains unaffected by β-blockers or inhibitors of dopamine β-hydroxylase (93–96). It is interesting that no s-IPSP response has been detected in the superior cervical ganglion (94) or inferior mesenteric ganglion of the guinea pig (98), in which SIF cells contain noradrenaline and/or adrenaline rather than dopamine (35), and that no distinct efferent synapses from SIF cells to ganglion cells have been identified in these ganglia (35).

Another effect of dopamine released from SIF cells is the modulation of the s-EPSP caused by muscarinic action of acetylcholine [Figure 2 (95, 97)]. This is a slow, long-lasting effect, which could be caused by liberation of dopamine from SIF cells some distance away from the ganglion cells (94) or, alternatively, by liberation of noradrenaline from the peripheral clusters of small granular vesicles in the perikarya of sympathetic nerve cells (23).

Recently, evidence has been obtained for the presence of an s-IPSP due to noradrenaline, probably released from axons, dendrites, or perikarya of noradrenaline-containing principal neurons (B. Libet, to be published).

CYCLIC AMP AS INTRACELLULAR MEDIATOR OF CATECHOLAMINE EFFECTS

An increase in the content of the cyclic AMP has been observed in the superior cervical ganglion of the rabbit under conditions similar to those resulting in s-IPSP: Preganglionic stimulation increases the cyclic AMP concentration up to about five

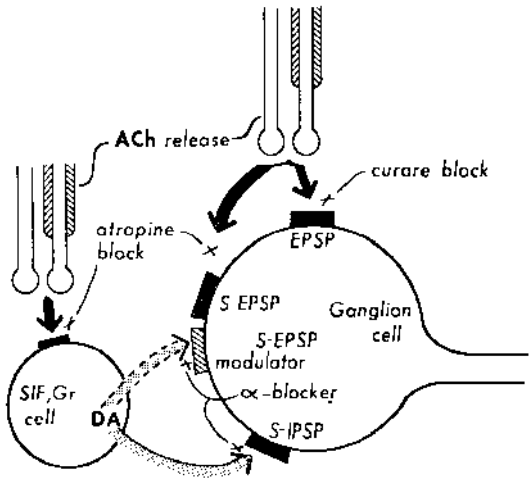

Figure 2 Schema for synaptic mediation of fast EPSP, s-IPSP, and s-EPSP. Although a separate postsynaptic receptor is shown for mediation of each action by dopamine, there is as yet no clear evidence available that excludes the possibility that one dopamine receptor mediates both actions. From Libet (95).

times that of control (99); muscarinic antagonist atropine and several α-adrenergic blockers prevent such increase, while nicotinic antagonist hexamethonium and β-blocker MJ 1999 do not (100); cholinergic agonists cause an increase in cyclic AMP (100); phosphodiesterase inhibitors potentiate the increase due to preganglionic stimulation or cholinergic agonists (100); dopamine causes a small but significant increase in the cyclic AMP content (100, 101); dopamine and several cyclic AMP derivatives cause similar hyperpolarization of the resting membrane potential of postganglionic neurons, and the phosphodiesterase inhibitor theophylline increases this hyperpolarization (101). In the bovine superior cervical ganglion, dopamine (or noradrenaline in high concentrations) causes a manyfold increase in the cyclic AMP content which is blocked by α-blocker phentolamine but not by β-blocker propranolol; the increased cyclic AMP has been histochemically demonstrated in the nerve cell bodies (102, 103).

All these observations can be taken to suggest that cyclic AMP is the intracellular mediator of the dopamine-induced s-IPSP (101, 103, 104). However, some other observations fit this view less perfectly. Only a 15% increase is caused by dopamine in the cyclic AMP content of the superior cervical ganglion of the rabbit, the ganglion with which most electrophysiological data have been obtained (see above). Preganglionic stimulation of the superior cervical ganglion of the rat, in whose SIF cells dopamine is the main amine, results in both s-IPSP (27) and increase in cyclic AMP (105) but exogenous dopamine causes much less increase in the cyclic AMP than isoprenaline, adrenaline, or noradrenaline and this increase is much more efficiently blocked by the β-blocker propranolol than the α-blocker phentolamine

(106, 107). Species differences in the fine structure of SIF cells and in the catecholamine-induced increase in cyclic AMP have recently been emphasized (61, 105). It is also possible that the main role of cyclic AMP is the long-lasting modulatory change in the muscarinic response to acetylcholine or the equivalent s-EPSP (97).

CONCLUSIONS

All SIF cells store large amounts of catecholamines in large granular vesicles, but different types of SIF cells store different catecholamines. There are afferent synapses from the lateral horn of the spinal cord on most SIF cells, but only some of these are interneurons being in efferent synaptic contact with principal neurons, other afferently innervated SIF cells apparently releasing their catecholamines by exocytosis to intercellular spaces and blood vessels. Other SIF cells yet are apparently chemoreceptors innervated by sensory synapses from nerve fibers of the glossopharyngeus nerve. No direct evidence of the participation of SIF cells in ganglionic transmission is available, but the electrophysiological phenomena of ganglionic transmission can best be explained by assuming that interneuronal SIF cells release catecholamines upon preganglionic muscarinic stimulation and that the catecholamines in turn affect appropriate receptors on the principal neurons, in which a slow inhibitory potential is generated, possibly through the action of cyclic AMP as an intracellular mediator.

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