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Compartmentalization of Monoaminergic Synaptic Vesicles in the Storage and Release of Neurotransmitter

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Abstract

Monoaminergic nerves are characterized by the presence of a population of small synaptic vesicles (40–60 nm in diameter) containing a few large vesicles (80–90 nm in diameter). Thus, although both types of vesicles contain monoamines, the small vesicles must be considered as the organoid responsible for the storage and release of the neurotransmitter, whereas the large ones possibly are involved in the modulation of the process. The small vesicles are electron-lucent or have an osmiophilic electron-dense core that is always linked to the vesicle membrane. Considering morphological and histochemical evidence under different experimental conditions, we proposed the existence of two compartments in the small vesicles: the core and the matrix, corresponding respectively to the electron-dense core and the electron-lucent space between the core and the vesicle membrane in osmium tetroxide fixations. The sizes of both compartments are inversely related, i.e., the smaller the core, the

larger the matrix and vice versa. The core even disappears, giving way to a small electron-lucent vesicle made exclusively by the matrix. Thus, the matrix is a constant component of the vesicle, whereas the core is a transient one. Each compartment has a different pool of amine: a loosely bound, easily releasable pool in the matrix and a tightly bound, more resistant pool in the core. These two pools subserve, respectively, a tonic or phasic release of the neurotransmitter, correlated with a tonic or phasic stimulation of the receptor. The core may be considered as a storage or reserve pool. Experimental evidence from our laboratory supports the concept that different mechanisms are operative in both compartments in the release of the neurotransmitter. For instance, a Ca^{2+}-independent release would be primarily concerned with the neurotransmitter contained in the matrix, and a Ca^{2+}-dependent efflux would be primarily related with the neurotransmitter stored in the core. However, it still must be established that a simple relationship exists between each kind of stimulus and each vesicle compartment, rather than both compartments being integrated in a dynamic functional unit.

Index Entries: Monoaminergic synaptic vesicles; synaptic vesicle compartmentation; vesicle pools of monoamines; storage of monoamines; monoamine release; regulation of monoamine release.

Introduction

The concept that neurotransmitters are stored in subcellular organelles emerged with the discovery of synaptic vesicles in electron microscopic studies (De Robertis and Bennett, 1955) and was further developed after their subsequent isolation by cell fractionation (De Robertis et al., 1962). The finding by von Euler and Hillarp (1956) that norepinephrine was present in a particulated fraction of bovine splenic nerves stimulated electron microscopists to identify these subcellular particles in monoaminergic nerves. Given that synaptic vesicles were discovered in cholinergic structures, they were considered at first, using osmium tetroxide fixations, as electronlucent organelles with uniform morphological and functional characteristics. Their uniform mean diameter was taken as the morphological counterpart of the quantal amount of neurotransmitter released by nerve stimulation (Fatt and Katz, 1952; De Robertis and Bennett, 1955; Katz, 1966).

It was demonstrated that this structural uniformity was not present in monoaminergic axons. In osmium tetroxide fixations, those nerves showed a "plurivesicular population" (De Robertis and Pellegrino de Iraldi, 1961; Pellegrino de Iraldi and De Robertis, 1961) made by two kinds of synaptic vesicles: granulated vesicles, which contain an osmiophilic electron-dense core, and electron-lucent vesicles similar to those found in cholinergic nerves. At first, this heterogeneity was not fully understood, but pharmacological, electrophysiological, and histochemical studies made under different experimental conditions have demonstrated that both granulated and electron-lucent vesicles are transient states of a unique organelle (Pellegrino de Iraldi et al., 1965; Pellegrino de Iraldi, 1980; Pellegrino de Iraldi, 1983).

Using glutaraldehyde before osmium tetroxide in fixation procedures, Hökfelt (1966) showed that monoaminergic vesicles could be classified in small (40–60 nm) and large (80–100 nm) synaptic vesicles. The latter are a very small proportion (2–10%) of the total vesicles (for refs, *see* Fried et al., 1981; Thureson-Klein, 1982). Both types of vesicles were described in monoaminergic axon terminals from the peripheral and central nervous systems (CNS) (De Robertis and Pellegrino de Iraldi, 1961; Pellegrino de Iraldi et al., 1963; Hökfelt, 1968). Small and large vesicles were also reported in the perikaryon (Eränkö, 1972) and in nerve trunks (Geffen and Livett, 1971; Tranzer, 1973; Thureson-Klein and Klein, 1990).

Small and large vesicles differ in biochemical constituents and physiological significance in neurotransmission (Hökfelt, 1966; Jaim Etcheverry and Zieher, 1969; Klein and Lagercrantz, 1982).

Even though monoamines have been localized in both, many lines of evidence support the concept that small vesicles are the organelles directly involved in the storage and release of neurotransmitters, in which the large ones possibly act to modulate the process. Both types of vesicles can release their contents by exocytosis (Thureson-Klein, 1983; Thureson-Klein and Klein, 1990). This review explores anatomical and functional organization of small vesicles, taking as a model the monoaminergic nerves of the rat pineal gland.

Morphological Organization of Small Monoaminergic Synaptic Vesicles

Monoaminergic synaptic vesicles were characterized from their first description (De Robertis and Pellegrino de Iraldi, 1961; Pellegrino de Iraldi and De Robertis, 1961), using osmium tetroxide fixations, by the presence of an electron-dense core separated from the vesicle membrane by an electron-lucent space (Fig. 1A). It was soon demonstrated by histochemical and pharmacological methods that the osmiophilic dense granules contained monoamines (Pellegrino de Iraldi et al., 1965; Pellegrino de Iraldi and Gueudet, 1969; Bloom, 1972). The uranaffin reaction for adenine nucleotides was also found to be positive in the core of monoaminergic vesicles (Richards and Da Prada, 1977), and a calcium binding site, modifiable by electrical stimulation and high potassium depolarization. was described at the level of the core (Pellegrino de Iraldi and Corazza, 1981a).

It is generally assumed that the core electron density in osmium tetroxide fixations is indicative of their catecholamine content (De Robertis and Pellegrino de Iraldi, 1961; Bloom and Barrnett, 1966; Van Orden et al., 1966; Hökfelt, 1968; Hökfelt and Jonsson, 1968; Bloom, 1972; Fillenz and Pollard, 1976; Tranzer and Richards, 1976). A great number of studies using a variety of agents that deplete or enhance dense core formation in vivo and in vitro have contributed greatly to this view (Pellegrino de Iraldi and De Robertis, 1961; Pellegrino de Iraldi et al., 1965; Tranzer and Thoenen, 1967; Pellegrino de Iraldi and Gueudet, 1969; Van Orden et al., 1976; Pellegrino de Iraldi and Suburo, 1971,1972a; Tomsig and Pellegrino de Iraldi, 1986,1987,1988). This assumption was sustained by the property of catecholamines to reduce O_sO_4 to O_sO_2 or metallic osmium in vitro thus providing the chemical basis for the *in situ* formation of electron-dense precipitates.

In vitro studies and pharmacological experiments strongly suggest that hydroxyl groups, mainly the phenolic ones, are responsible for the reducing capacity of the amines (Hökfelt, 1971). According to these indications, the osmiophilia would require the presence of the two phenolic groups. The reaction between glutaraldehyde and amines does not block the phenolic hydroxyl groups (Pearse, 1980) and the osmiophilia persists.

Even if a correlation between osmiophilia and monoamine content is apparent, in many cases the granularity induced by osmium tetroxide does not always reflect the monoamine content of the vesicles. Iwayama and Furness (1971) observed in the guinea pig vas deferens an increment of the vesicle osmiophilic granulation after prolonged incubation in a modified Krebs' solution. However, using fluorescence histochemical methods (Falck, 1962) no differences were observed in treated and untreated tissues. In the CNS, small vesicles do not show an osmiophilic electron-dense core in O_sO_4 fixation, and a stronger oxidant is needed to visualize electron-dense vesicle cores (Hökfelt, 1971). It can be surmised that at least in some cases, the neurotransmitter within the vesicles is in a state that makes it unable to react with osmium tetroxide.

An electron-dense osmiophilic core may be visualized in the small synaptic vesicles of the rat pineal nerves using a fixative containing a high concentration of $CaCl_2$ (50–90 m*M*) after the depletion of monoamines by collidine (Pellegrino

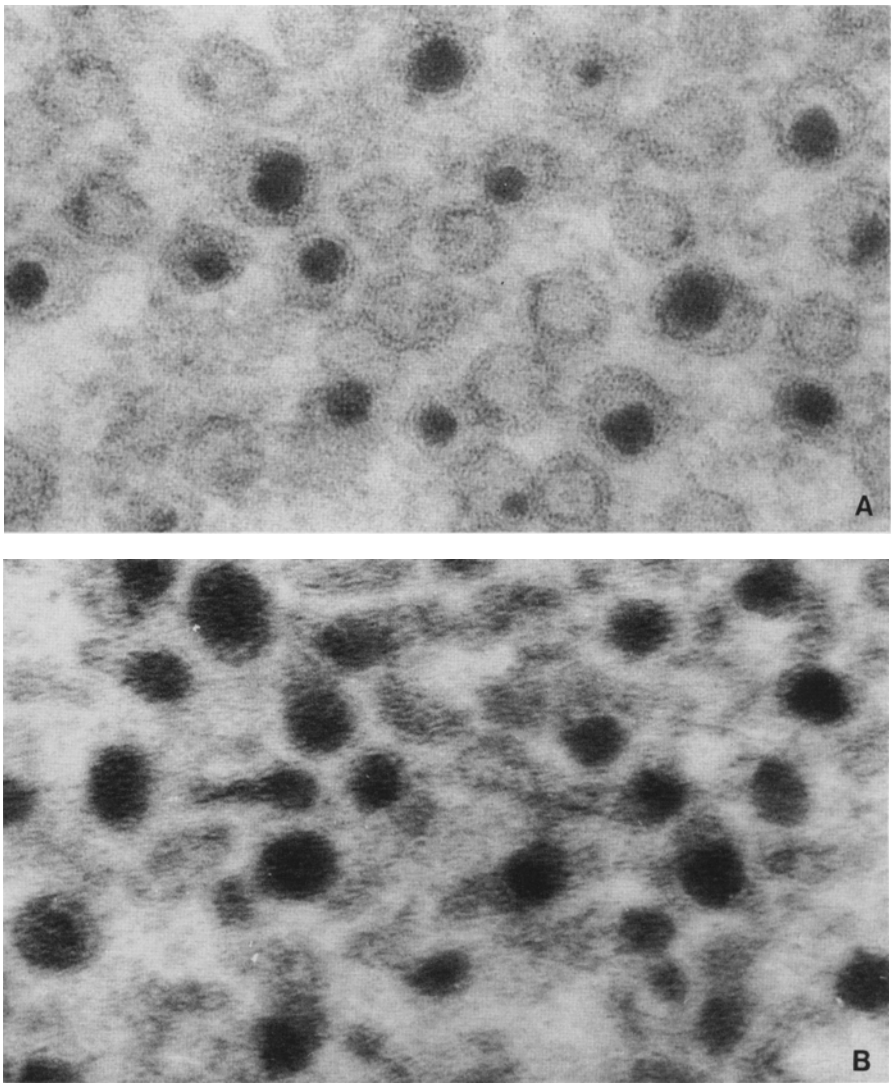

Fig. 1. Monoaminergic synaptic vesicles from rat pineal nerves. A: Without previous treatment, fixed in osmium tetroxide. Electron lucent and granulated vesicles are seen. The osmiophilic electron-dense cores of granulated vesicles are of variable size, and the matrix or electron-lucent space between the core and the vesicle membrane may be appreciated. B: After the administration of 5-OH-DA and glutaraldehyde-osmium tetroxide fixation. Many vesicles, totally stained, are seen, and the matrix is not discernible.

de Iraldi and Corazza, 1981a). In cholinergic vesicles, which are electron-lucent after the osmium tetroxide conventional fixations, an electron-dense granule may be seen when fixatives containing a high concentration of $CaCl_2$ are used (Boyne et al., 1974; Politoff, 1974; Pappas and Rose, 1976; Iraldi and Pellegrino de Iraldi, 1987). In these cases, osmiophilic electron-dense cores seem to be the result of a calcium deposit.

Fried et al., (1981) found that in small noradrenergic vesicles from rat seminal ducts, both noradrenaline, and to a lesser extent other matrix materials, form electron-dense cores in the presence of magnesium and adenosine-

triphosphate and furthermore that noradrenaline is more important for dense core formation in small vesicles than in the large ones. The clear space was disregarded or considered an artifact of the fixation techniques. The systematic study of small synaptic vesicles in different experimental schedules led us to propose that the clear space is an important component of the vesicles that are formed by two compartments, the dense core and the clear space, which we called the matrix (Pellegrino de Iraldi and Suburo, 1971, 1972a,b; Pellegrino de Iraldi, 1980,1983). According to that proposal, each compartment has a different functional significance in the synthesis, storage, and release of the neurotransmitter. The vol of the two compartments is inversely related: i.e., the smaller the core, the larger the matrix and vice versa. The core may be absent, resulting in vesicles formed exclusively by the matrix. Thus, the matrix is a constant component of the small vesicles and the core is a transient one. The relative frequency of granulated and clear vesicles is variable in different territories. Under conditions of rest, core vesicles are scarce in organs with a predominant "tonic" functioning (e.g., small arteries) and abundant in organs with an important "phasic" functioning (e.g., the vas deferens and the pineal gland). It has been demonstrated that this relative frequency may be modified by pharmacological treatments or other stimuli.

Within each nerve terminal, the size and density of the vesicle cores are not uniform. It can be speculated that this heterogeneity is probably the morphological expression of a functional asynchronism of the synaptic vesicles (Pellegrino de Iraldi and Corazza, 1983c). A metabolic intravesicular heterogeneity has been demonstrated for cholinergic vesicles, by means of fractionation and biochemical methods; this phenomenon has been incorporated into the formulation of a model for the release of the neurotransmitter by exocytosis (Zimmermann, 1982). The core is always linked to the vesicle membrane, being directly in contact or connected to it by a pedicle (Pellegrino de Iraldi and Suburo, 1972b). This suggests that the formation of the core is dependent in some way on a special locus of the vesicle membrane.

When a mixture of zinc iodide and osmium tetroxide (ZIO) was used, a reverse image of that seen with osmium tetroxide was obtained, and the matrix appears denser than the core (Pellegrino de Iraldi and Gueudet, 1968). The ZIO reaction was shown to be a result of the presence of reactive -SH groups that possibly belong to protein molecules within the vesicles (Pellegrino de Iraldi, 1975; Reinecke and Walther, 1978). The ZIO reaction is temperature- and time-dependent and varies in different types of synaptic vesicles (Pellegrino de Iraldi, 1977). In monoaminergic vesicles, it is affected by drugs interfering with the uptake, storage, and synthesis of monoamines (Pellegrino de Iraldi and Suburo, 1971; Pellegrino de Iraldi and Cardoni, 1979). It also has been shown that tyramine affects the ZIO reaction differently in the matrix and the core (Pellegrino de Iraldi and Suburo, 1972a).This finding supports the idea that the matrix and the core are different compartments of the small vesicles.

Two Pools of Amines in Monoaminergic Small Synaptic Vesicles

Studies made in our laboratory (Pellegrino de Iraldi, 1980; Pellegrino de Iraldi and Corazza, 1981b) have shown that endogenous amines are distributed, in small monoaminergic synaptic vesicles, in two pools that can be characterized by histochemistry and electrical stimulation: a loosely bound pool located in the matrix and a tightly bound pool located in the core. The behavior of both pools, which were observed under stimulated and rested conditions, is compatible with the notion that amines stored in the core form a reserve pool, whereas amines stored in the matrix correspond to a functional pool that can be released "spontaneously," and with electrical stimulation being replaced by the newly

synthesized transmitter or by the amines stored in the core.

A similar distribution is adopted by the false transmitter formed after the administration of 5-hydroxydopamine (5-OH-DA) or by its precursor 5-hydroxy DOPA. The behavior of the false transmitter may be easily followed with an electron microscope because of the intense osmiophilia induced by its storage in synaptic vesicles (Tranzer and Thoenen, 1967). We observed that, as reported by these authors, the osmiophilia induced by 5-OH-DA could occupy the entire vesicle, matrix, and core (Fig. 1B) after glutaral-dehydeosmium tetroxide fixation but was restricted to the core when osmium tetroxide was used alone. In this case, the image was similar to that of the untreated control shown in Fig. 1A, which suggests that the false neurotransmitter was bound differently in the matrix and the core (Pellegrino de Iraldi and Corazza, 1983a) as previously reported using another experimental schedule (Pellegrino de Iraldi, Gueudet, and Suburo, 1971).

In our laboratory, we have studied the release of the false transmitter from the rat pineal nerves in vivo, at rest, and under electrical stimulation (Pellegrino de Iraldi, 1980; Pellegrino de Iraldi and Corazza, 1981b) in animals treated according to Tranzer and Thoenen (1967): seven doses of 20 mg/k of 5-OH-DA were injected ip over a period of 48 h at 8-h intervals. One hour after the last injection, most of the vesicles appear to be filled with osmiophilic material. Five hours after the last injection, the osmiophilia was restricted to the core and the matrix was electron-lucent. Electrical stimulation applied 1 or 5 h after the last injection to the afferent trunks of both superior cervical ganglia (sq pulses of 1 msec, 25 V, 25 Hz for 30 min) depleted the osmiophilic material from the matrix and the core. Thus, the false transmitter stored in the matrix was preferentially depleted at rest and the electrical stimulation acted on both compartments.

The release of the false transmitter by high potassium depolarization was also studied in vitro (Pellegrino de Iraldi, 1983). It was observed that a high potassium concentration in the absence of calcium in the extracellular medium preferentially releases the false transmitter stored in the matrix and that calcium was required for depletion of the core. The osmiophilic cores of noninjected rats were reduced in size and frequency by electrical stimulation but reduced only in size by high potassium in the absence of calcium (Pellegrino de Iraldi, 1983). It is interesting in this context that the osmiophilia of synaptic vesicles of rats treated with 5-OH-DA represents a pool of amines recently formed, whereas the core osmiophilia of uninjected rats represents a pool less recently stored in the vesicles.

The finding of two pools of amines in the small synaptic vesicles of rat pineal nerves, here reviewed, seems to be in conflict with data obtained from the small vesicles in the vas deferens by Fried (1980), who reported kinetic evidence for a single pool. Different reasons could explain this discrepancy. The most important of them is perhaps that our studies were made in intact tissues, whereas Fried used isolated vesicles obtained after homogenization and differential centrifugation. In the procedure, noradrenaline may be lost in parallel with nonamine components of the vesicles (Thureson-Klein, 1982). Studies now in progress in our laboratory also support this view (Pellegrino de Iraldi, Corazza, and Tomás, unpublished). Furthermore, it may be of importance that different tissues and experimental conditions are employed in both cases to study the release of neurotransmitter.

The behavior of large vesicles was not studied in our publications here reviewed, but two pools of amines have been reported (Klein, 1973; Klein and Lagercranz, 1982) in the large noradrenergic vesicles of vas deferens nerves, one rapidily and one slowly depletable pool. Two pools of catecholamines also have been described in the chromaffin granules of the adrenal medulla: one exchangeable, with a rapid release rate, and one nonexchangeable, with a very slow release rate (Slotkin et al., 1971).

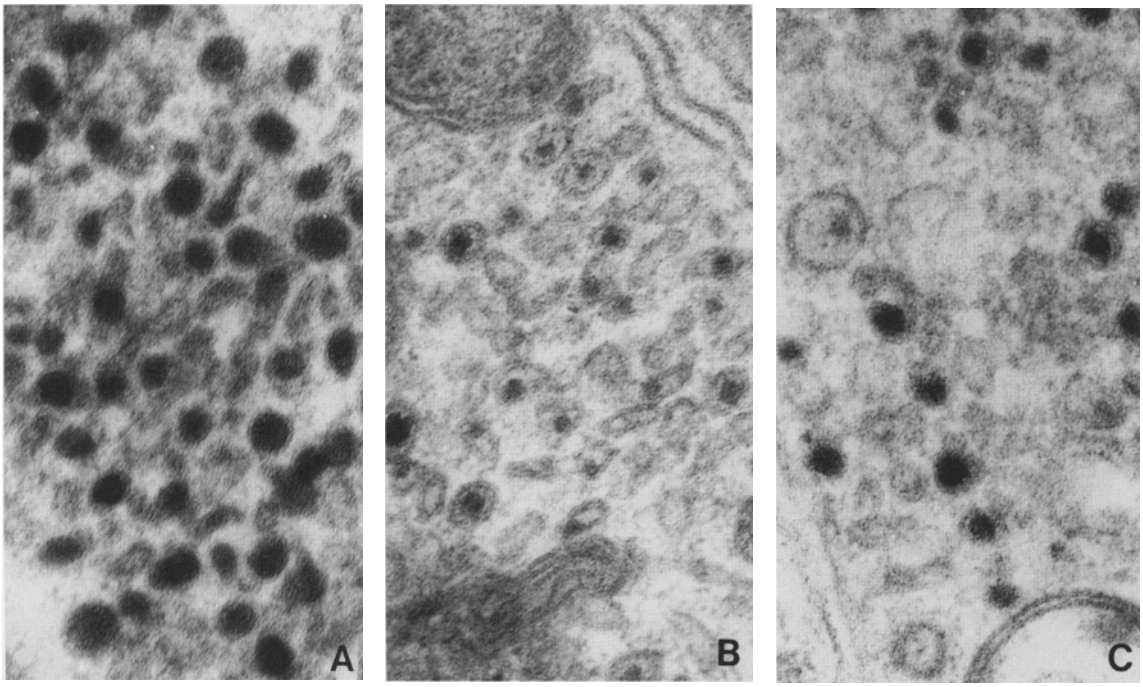

Fig. 2. Influence of the electrical stimulation on the uptake, storage, and release of 5-OH-DA in rat pineal nerves. Glutaraldehyde osmium tetroxide fixation. A: Control injected and nonstimulated. Many synaptic vesicles are totally stained. B: The drug was injected just at the end of stimulation. Many vesicles have an electron-dense core. Totally stained vesicles are not seen. C: The drug was injected 1 h after stimulation. The proportion of synaptic vesicles totally stained is significantly lower than in nonstimulated controls (A).

Influence of Electrical Stimulation on Uptake, Storage, and Release of 5-OH-DA in Synaptic Vesicles

Electrical stimulation affects the subsequent uptake, storage, and release of the false neurotransmitter formed by the administration of 5-OH-DA (Pellegrino de Iraldi and Corazza, 1983a,b, and unpublished results). Electrical stimulation was administered to the afferent trunks of both superior cervical ganglia of adult rats for 20 min (sq pulses of 1 msec, 25 Hz, 25V) under chloral hydrate anesthesia, with 5-OH-DA administered (30 mg/k ip) 10 min before the end of stimulation, just at the end of stimulation, and 5, 10, 30, and 60 min after stimulation was terminated. The animals were killed 30 min after the last injection. Nonstimulated injected and non-injected rats were used as controls. Fixation was done in glutaraldehyde-osmium tetroxide. In nonstimulated injected controls, most of the vesicles (Fig. 2A) were filled with osmiophilic electron-dense material or had a prominent electron-dense core.

When the drug was administered during stimulation, many synaptic vesicles showed a prominent electron-dense core, but totally stained vesicles were absent or very scarce. The dense cores were larger, denser, and more abundant than in the synaptic vesicles of noninjected rats. An essentially similar picture was evident when the drug was administered immediately after stimulation was terminated (Fig. 2B). Totally stained vesicles reappeared progressively as time elapsed between stimulation and injection. Even

in animals injected 1 h after the stimulation (Fig. 2C), the proportion of totally stained vesicles was significantly less than those formed in nonstimulated animals.

The decrease in the matrix osmiophilia could be explained by an impairment of the uptake, by alteration in the storage mechanism(s) or by an enhanced release. The fact that the dense-cores were more abundant, denser, and larger in stimulated injected animals than in noninjected ones seems to indicate that stimulation does not prevent monoamine uptake and/or storage and points to an acceleration of the release of the neurotransmitter from the matrix. This interpretation is compatible with the knowledge that electrical stimulation releases catecholamines and simultaneously accelerates their synthesis in such a way that the total content of amines in a given organ is only moderately decreased (Luco and Goñi, 1948; von Euler and Bjorkman, 1955; Gordon et al., 1966; Molinoff et al., 1970; Thoenen, Mueller, and Axelrod, 1970).

The persistence of changes induced in the storage of the false transmitter after stimulation was terminated seems to indicate that a plastic modification is taking place in the vesicles and that this change is prominent in the matrix. This is supported by the fact that the most important change occurs at this level, as shown by the modification of the proportion of totally stained vesicles. Vesicle modification could thus be involved in processes related to a variation in the amount of neurotransmitter release. An example of this occurs during learning, when the number of quanta of neurotransmitter released by cells is modulated at the presynaptic level (Kandel and Schwartz, 1982). Our results suggest that the rate of transmitter release may be regulated at least in part at the level of the synaptic vesicles themselves and that the core and the matrix have different roles. These results are in line with the acceleration of miniature end plate potentials for a period following tetanic stimulation of the nerve at the neuromuscular junction and neuroneuronal synapses (Miledi and Thies, 1971).

Selective Effect of Ouabain on Neurotransmitter Pools of Monoaminergic Synaptic Vesicles

The physiological role of membrane Na^+K^+-activated adenosine 5'-triphosphatase (Na^+K^+-ATPase, EC 3.6.1.3) in the regulation of the neurotransmitter release process was first reported by Paton et al. (1971). Inhibition of the enzyme increases the release of neurotransmitters, whereas stimulation has the opposite effect. Ouabain, a potent and specific Na^+K^+-ATPase inhibitor, potentiates release of several neurotransmitter substances (Vizi, 1978; Fairbrother et al., 1990). In our laboratory, the effect of ouabain on rat pineal nerves was studied in experiments made in vitro (Pellegrino de Iraldi and Rodríguez de Lores Arnaiz, 1989). The effect of the drug, dissolved in a calcium-free tyrode solution, was assayed on glands of animals injected with 5-OH-DA 30 min before decapitation and in noninjected rats. After incubation, the pineals of injected rats were fixed in glutaraldehyde-osmium tetroxide, and the glands of uninjected animals were directly treated with osmium tetroxide or with a chromaffin reaction for catechol and indoleamines, according to Wood (1967). It was observed that the osmiophilia induced by the administration of 5–OH-DA and revealed by glutaraldehyde-osmium tetroxide fixation was specially reduced at the level of the matrix (Fig. 3A,B). The direct osmiophilia and the chromaffin reaction of synaptic cores of pineal nerves from uninjected rats was affected little or not at all (not shown). Thus, a differential effect of the drug on both vesicle compartments is evident. These results also support the concept that different mechanisms are involved in the release of neurotransmitters located in the synaptic vesicle compartments. In this context, it must be mentioned that an endogenous inhibitor of Na^+K^+-ATPase, which does not inhibit other enzymes of synaptosomal membranes, also produces a differential effect on the neurotransmitter pools of pineal nerve synaptic

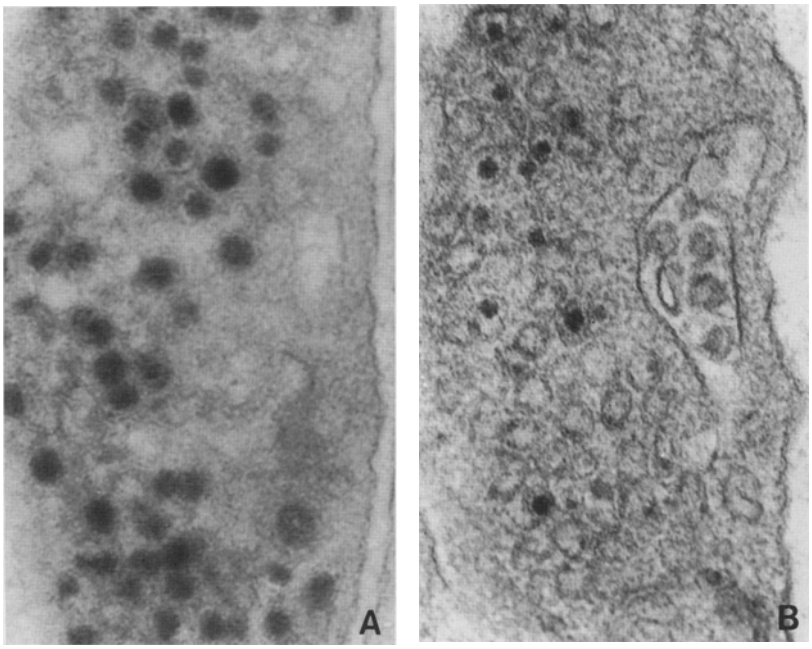

Fig. 3. Effect of ouabain on the storage and release of the false neurotransmitter induced by the administration of 5-OH-DA. Fixation in glutaraldehyde-osmium tetroxide. A: Control injected and incubated in tyrode solution without calcium. B: Pineal nerves of a rat injected with 5-OH-DA after the incubation in a calcium-free tyrode solution containing ouabain. Totally stained vesicles are not seen; electron-dense cores are evident.

vesicles (Rodríguez de Lores Arnaiz and Pellegrino de Iraldi, 1989).

Effect of Pyridine and Its Methylated Derivatives on Storage and Release of Monoaminergic Neurotransmitters

Pellegrino de Iraldi and Corazza (1981a) demonstrated that the osmiophilia of synaptic vesicles in rat pineal nerves disappears when collidine (2-4-6-trimethylpyridine) is used as the buffering substance in fixation procedures. This phenomenon does not occur with other commonly used buffers, e.g., cacodylate or phosphate. This finding was further investigated in the pineal gland (Tomsig and Pellegrino de Iraldi, 1987) and vas deferens (Tomsig and Pellegrino de Iraldi, 1986) nerve endings. It also was found that collidine buffer renders negative not only the osmiophilia but also the chromaffin reaction of the synaptic vesicle cores. This abolition was apparent when fresh tissues were briefly incubated in collidine or when they were fixed in glutaraldehyde-osmium tetroxide alone, using collidine as a buffer substance. These and other results strongly suggest that the histochemical effect was a result of depletion of monoamines stored in the core. To examine this hypothesis, the effect of collidine on tissues that have taken up tritiated noradrenaline was studied (Tomsig and Pellegrino de Iraldi, 1988). It was found that tritium was released very rapidly to the incubation medium when collidine was applied to fresh tissues. This effect was not observed with other buffers such as cacodylate or phosphate. It was found that tritium release also occurred, although to a lesser extent, when

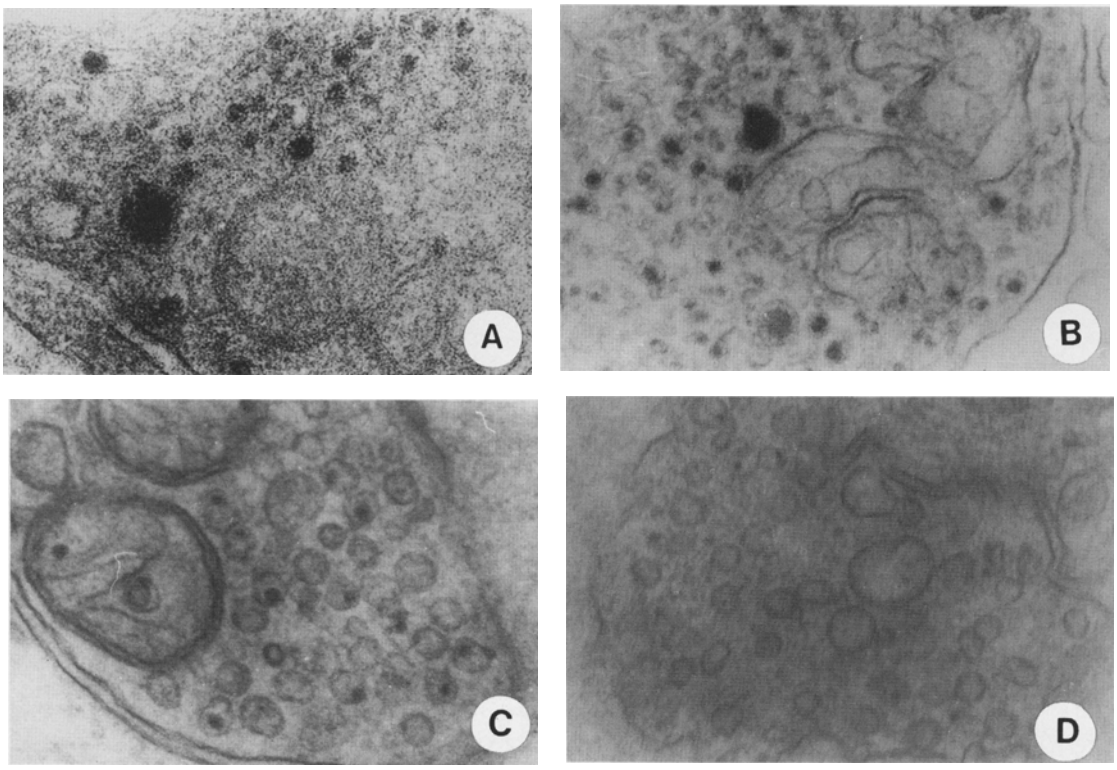

Fig. 4. Effect of pyridine on the pineal nerves of untreated rats in experiments made in vitro. A and B: Fixation in glutaraldehyde-osmium tetroxide. C and D: Fixation in osmium tetroxide alone (direct osmiophilia). A and C incubated in Krebs' solution. B and D incubated in Krebs' solution containing pyridine. Direct osmiophilia (D) was abolished, whereas postglutaraldehyde osmiophilia of the electron-dense cores remained.

tissues were fixed in glutaraldehyde or osmium tetroxide, using collidine as a buffer, and that this release was not significant when collidine was applied to previously fixed tissues. Paper chromatographic analysis showed that the radioactive compound(s) extracted from tissues by collidine corresponded to noradrenaline or closely related compounds.

More recently, the effect of collidine was compared with the effect of pyridine and its mono- and dimethylated derivatives (Pellegrino de Iraldi et al., 1991). It was observed that lutidine (2-6-dimethylpyridine) abolished the osmiophilia of fresh tissues (direct osmiophilia) and the osmiophilia post glutaraldehyde and strongly diminished the chromaffin reaction. Picolines (2- and 4-monomethylpyridine) and pyridine only abolished direct osmiophilia. The effect of pyridine is shown in Fig. 4. The release of ^{3}H-noradrenaline previously taken up induced by the Kreb's solution was increased 70-fold by collidine, as previously reported (Tomsig and Pellegrino de Iraldi, 1988) and was enhanced 27-fold by lutidine, 9-fold by picolines, and 7-fold by pyridine. These results indicate that the methylation of the pyridine ring increases its effect on the release of monoamine transmitter and furthermore indicate that this increase is proportional to the number of methyl groups. They algo suggest that direct osmiophilia might visualize a more labile pool in the storage site of monoaminergic synaptic vesicles than the glutaraldehyde-osmium tetroxide fixation or the chromaffin reaction.

It has been demonstrated that collidine strongly inhibits $Na^{+}K^{+}$-ATPase (Rodríguez de Lores Arnaiz and Pellegrino de Iraldi, 1990).

However, the fact that ouabain, which inhibits the enzyme, does not affect the osmiophilia and the chromaffin reaction of the cores suggests that the collidine effect is a complex phenomenon in which a mechanism(s) in addition to inhibition of Na^+K^+-ATPase are involved. Studies in progress in our laboratory (Pellegrino de Iraldi, Corazza, and Tomás) take into account the inhibition of Na^+K^+-ATPase by collidine (Rodríguez de Lores Arnaiz and Pellegrino de Iraldi, 1990) and the effect of ouabain previously mentioned.

Conclusions

The information here reviewed—based on evidence derived from morphological, pharmacological, biochemical, histochemical, electrical stimulation, and high potassium depolarization studies—indicated that small monoaminergic synaptic vesicles can be considered to be formed by two compartments: the matrix and the core. Each compartment has a different pool of monoamines: a loosely bound, easily releasable pool in the outer compartment, called the matrix, and a tightly bound, more resistant pool in the central compartment, called the core. These two pools subserve primarily a tonic or phasic stimulation of the receptor. The monoamines synthesized or taken up by the matrix may be released or stored in the core. The amines stored in the core may be released to the matrix.

The postulation that the neurotransmitter is synthesized in the vesicle matrix is still a working hypothesis. The presence in the small synaptic vesicles of dopamine-β-hydroxylase, the enzyme that catalizes the conversion of dopamine to noradrenaline, is controversial. The presence (Bisby et al., 1973; Nelson and Molinoff, 1976; Rush et al., 1978; Neuman et al., 1984; Schwarzenbrunner et al., 1990) and the absence of the enzyme (Chubb, De Potter, and De Schaepdryver, 1970; Klein and Thureson-Klein, 1984; Willems and De Potter, 1983; Lara and Belmar, 1989) have been reported. Klein et al. (1979) have called attention to the possibility of artefactual conclusions regarding subcellular compartments on the basis of density gradients. It is interesting in this context that immunohistochemical results obtained in experiments on the rat iris suggest that dopamine-β-hydroxylase also is present in small synaptic vesicles (Rush et al., 1978).

According to the calcium hypothesis (Katz and Miledi, 1967; Blaszkowsky and Bogdanski, 1971; Llinás et al., 1976; Rahamimoff, 1977), synaptic transmission is fundamentally controlled after depolarization by a rapid increase in the axoplasmic calcium concentration determined by calcium entering from the extracellular space or released from the intracellular stores. The release of the neurotransmitter at rest is less calcium-dependent (Katz, 1966). Elevated potassium pulses may produce release of the neurotransmitter in the absence and presence of calcium in the extracellular fluid, and a calcium-dependent and calcium-independent release of the neurotransmitter are distinguished (Haycock et al., 1978).

The effect of ouabain in the absence of calcium and the release of the neurotransmitter at rest suggest that the release of the neurotransmitter contained in the matrix does not require the presence of calcium, whereas the release of neurotransmitter stored in the core is a calcium-dependent phenomenon. However, a simple relationship between a kind of stimulus and each vesicle compartment must be excluded. Thus, electrical stimulation and high potassium depolarization in the presence of calcium affect both compartments. Furthermore, high potassium in the absence of calcium induces a gradual reduction in the size of the cores, suggesting a progressive release of the stored amines. Thus, two different but interlocked mechanisms for release of the neurotransmitter would be the physiological counterpart of the anatomical duality of small monoaminergic synaptic vesicles. A functional duality of both compartments is also evidenced by their behavior in the uptake, storage, and release of a false transmitter after electrical stimulation. The effect of pyridine on the

histochemistry of the core compartment suggests that more than one pool of the neurotransmitter are located in it.

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