The Natural History of the Chromaffin Cell — Twenty-five Years on the Beginning

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Summary. Aspects of the functional anatomy of the chromaffin cell since 1950 are reviewed—beginning with the identification of noradrenaline in the adrenal gland and the first descriptions of distinct adrenaline- and noradrenaline-storing cells.

Reference is made to the identification of specific proteins and neuropeptides in chromaffin cells, of the storage of endogenous and exogenous amines including 5HT.

The influence of corticosteroids and nerve growth factor on cell phenotypes is discussed and a plea made for the specific use of descriptive terms with due regard to historical and functional context: this relating particularly to the appropriate labelling of elements as SIF cells.

The review includes reference to recent quantitative studies on the rat adrenal medulla and adrenal chromaffin cells and to the innervation of the adrenal medulla by pre- and postganglionic sympathetic neurons, by spinal afferent neurons and by sensory and motor vagal fibers.

In 1950 it appeared from world literature that the chromaffin cell was a neglected field of research, and had been so since the turn of the century. Nevertheless the publication of EULER and HAMBERG (1949) on the presence of L-noradrenaline in the adrenal medulla clearly indicated the need for reappraisal. This view must have been shared by the late Nils-Åke HILLARP in Lund and the late Olavi ERÄNKÖ in Helsinki—though their interests were unknown to me at the time.

In 1950 BÄNDER, using histological techniques described two types of chromaffin cells in the mouse, cat and dog adrenal medulla. Two years later ERÄNKÖ (1952) distinguished two types in the rat using fluorescence microscopy after formaldehyde fixation. In 1953 HILLARP and HÖKFELT introduced the iodate reaction for the identification of noradrenaline-storing cells and correlated the relative number of

iodate-positive cells in the adrenal medulla with adrenaline/noradrenaline content.

Early attempts to separate the subcellular amine storage particles by centrifugation of homogenates of adrenal medulla in isotonic sucrose had been frustrated by the fact that they sedimented with mitochondria (BLASCHKO and WELCH, 1953). However, membrane-bound chromaffin granules were clearly identified in chromaffin cells by LEVER (1955) by using one of the recently introduced electron microscopes. Later, BLASCHKO et al. (1957), separated mitochondria and chromaffin granules using a sucrose gradient and noted their high adenosine triphosphate (ATP) content.

The first complete analysis of the chemical composition of bovine chromaffin granules was published by HILLARP (1959) and HILLARP and THIEME (1959) reported on the total nucleotides present in chromaffin granules.

It is now 24 years since the manuscript of my monograph on "The Natural History of the Chromaffin Cell" (COUPLAND, 1965a) went to the publishers. The time coincided with the identification of the adrenaline (A)- and noradrenaline (NA)-storing chromaffin cells (COUPLAND et al., 1964; COUPLAND, 1965b, c; COUPLAND and HOPWOOD, 1966) at the ultrastructural level, following initial tissue fixation by glutaraldehyde followed by osmication.

SELECTED SUBSEQUENT MILESTONES

Early observations of HILLARP (1958a, b) had drawn attention to the presence of ATPase in the membrane of the chromaffin granule, the concomitant discharge of ATP and catecholamines from the insulin-stimulated sheep adrenal gland and to the presence of catecholamines and ATP in a molar ratio of 4:1 in the chromaffin granules. Further observations on the

Mg⁺⁺ATP-dependent storage mechanism of catecholamine and serotonin were published by CARLSSON et al. (1963).

Peter BANKS (1965, 1966) working with Hermann BLASCHKO in Oxford confirmed the findings of the Swedish group and BANKS and HELLE (1965) reported that protein as well as catecholamines was secreted from the perfused isolated bovine gland after appropriate stimulation. They suggested that the loss of protein may be the consequence of the fusion of the membrane of the chromaffin granule with the cell surface membrane, as had initially been suggested by DE ROBERTIS and SABATINI (1960) and first illustrated by COUPLAND (1965a, b).

The soluble proteins of the chromaffin granules were collectively named the chromogranins by BLASCHKO et al. (1967). Subsequently the soluble proteins have been shown to include a substantial proportion of the granule content of dopamine-B hydroxylase, enkephalin precursors and glycoprotein III as well as the major acidic proteins rich in glutamic acid and still usually referred to as chromogranins A, B and C (WINKLER, 1976; WINKLER et al., 1986). Recently a change in nomenclature has been proposed (EIDEN et al., 1987) with chromagranin A, (the major component in bovine chromaffin granules) retaining its name as does chromagranin B, (the predominant form in rat and human chromaffin granules and sometimes referred to as secretogranin I). Chromogranin C, a minor component of bovine chromaffin granules but like chromagranins A and B widely distributed in endocrine and nervous tissue is designated secretogranin II.

The importance of calcium ions in the secretory response of chromaffin cells to acetylcholine, was noted by DOUGLAS and RUBIN (1961). Subsequently DOUGLAS (1966) proposed a Ca⁺⁺ dependent mechanism of stimulus-secretion coupling for the exocytotic release of catecholamine from chromaffin cells and for oxytocin and vasopressin from the posterior pituitary. He suggested that the granule membrane initially incorporated into the plasma membrane was subsequently retrieved in the form of microvesicles.

The concept of exocytosis was further supported by evidence of the simultaneous loss of dopamine-B-hydroxylase, ATP and catecholamines (VIVEROS et al., 1968, 1969) and the co-release of enkephalins and catecholamines from cultured chromaffin cells (VIVEROS et al., 1979; LIVETT, 1981). Essential morphological evidence of exocytosis including membrane exposure, retrieval and recycling was provided by Hans WINKLER and colleagues using freeze-

etching (SMITH et al., 1981) and immunohistochemistry (SCHMIDT et al., 1983; PATZAK and WINKLER, 1986).

Even so the molecular mechanism is still unclear as are the roles of calmodulin, actin, actinin, myosin, G proteins and synexin (BURGOYNE, 1984, 1987; FOURNIER and TRIFARO, 1988).

Excellent reviews on the anatomy and molecular composition of the chromaffin granule have been published by WINKLER and colleagues (WINKLER, 1976; WINKLER et al., 1986; WINKLER and CARMICHAEL, 1982).

Catecholamine synthesis and storage

During the period since 1965 KIRSCHNER's observations (KIRSCHNER, 1957; KIRSCHNER and GOODALL, 1957) on the cellular location of enzymes concerned with catecholamine synthesis have been confirmed in particular by SABBAN and GOLDSTEIN (1984). Phenylethanolamine N-methyltransferase (PNMT) and tyrosine hydroxylase (TH) are located in the cytosol and dopamine B-hydroxylase (DBH) in chromaffin granules. Recently JOH et al. (1983) has suggested the possibility of homologous gene coding regions for the enzymes, and HELLE et al. (1984) suggested that soluble DBH may be derived from a membrane precursor by the action of proteolytic enzymes.

The intracellular location of recently synthesized catecholamines *in vivo* was demonstrated by COUPLAND and KOBAYASHI (1976) and COUPLAND et al. (1976) by electron microscopic autoradiography and assay following the administration of ³H-L-DOPA. Tritium labelled catecholamines were always associated with chromaffin granules or the immediately adjacent cytosol and not with Golgi complex or rough endoplasmic reticulum. Furthermore, the labelling of chromaffin granules occurred simultaneously throughout the cell. Thus no support was obtained for the concept of recently synthesized catecholamines charging only recently formed granules. Labelled amines were more rapidly lost from A cells than from NA cells. These findings were subsequently confirmed in the rat (BENCHIMOL and CANTIN, 1982).

Amine uptake by chromaffin cells

Evidence for an ATP-ase located in the membrane of chromaffin granules and an Mg⁺⁺ATP-dependent storage mechanism was presented by HILLARP (1958a) and CARLSSON et al. (1963). Subsequent work on the proton translocating ATPase has been discussed at length by WINKLER et al. (1986) as well as evidence for a specific carrier, which transports not

only A, NA and DA but also 5-hydroxytryptamine, tyramine and isoprenaline across the granule membrane; they also discussed evidence for a nucleotide transport system and carrier in chromaffin granules.

Uptake of exogenous catecholamines and 5-hydroxytryptamine (5HT)

Evidence for a zonal distribution of [^3H] dopamine, [^3H] NA and [^3H] A in the adrenal medulla following intravenous injection of the amines was presented by HIRANO et al. (1977) and KENT and COUPLAND (1981) and its dependence on pituitary ACTH was reported by HIRANO and KOBAYASHI (1978).

A similar zonal pattern is observed after the intravenous injection of [^3H] 5HT (KENT and COUPLAND, 1984) and A cells were again more heavily labelled than NA cells. Unlike catecholamines [^3H] 5HT did not label adrenergic nerve terminals.

A striking difference in the loss of radioactivity after intravenous administration of [^3H]NA and [^3H] 5HT was noted. After 7 days the [^3H]NA labelling of A cells was only *c* 10 per cent that on day-1 while [^3H]5HT showed only an insignificant decrease. Since both amines are stored in the chromaffin granules (CARLSSON et al., 1963) the differential loss raises the possibilities of either some loss of catecholamines by mechanisms other than by exocytosis or the existence of a more effective reuptake mechanism for 5HT.

The coexistence of 5HT and catecholamines in the adrenal medulla

Immunohistochemical and biochemical investigations have demonstrated the presence of small amounts of 5HT in A cells of the rat adrenal medulla (VERHOFSTAD and JONSSON, 1983; HOLZWORTH and BROWN-FIELD, 1985). Is the 5HT synthesized locally or taken up from the circulation? Our observations (KENT and COUPLAND, 1984) are in keeping with the latter suggestion.

Ontogenesis of chromaffin cells

Recent advances in our knowledge and understanding of the developmental origin and plasticity of chromaffin cells, owe much to the elegant work of Nicole LE DOUARIN and colleagues (LE DOUARIN, 1980, 1982) using chick-quail chimeras *in vivo*, *in vitro* and growing combined tissues on the avian chorioallantoic membrane. *In vitro* techniques for the study of the differentiation of mammalian sympatho adrenal tissue date back to HERVONEN and KANERVA (1973) and major contributors include UNSICKER,

PATTERSON and TISCHLER and co-workers.

The neural crest origin of chromaffin cells is now beyond doubt. In the mammalian fetus sympatho-chromaffin cells invade the medial side of the adrenal cortical anlage to form the adrenal medulla. In the rabbit both primitive sympathetic cells and pheochromoblasts can be identified by day-14 (COUPLAND and WEAKLEY, 1968) while in the prenatal rat similar granules have been seen on day-12 (ELFVIN, 1967). Initially granule cores are highly electron dense, suggesting NA-storage, and are often peripherally distributed. As cell differentiation proceeds granules increase in number and size and the elongated nucleus typical of the pheochromoblast takes on a more rounded form. Both NA and A-storage granules have been identified in rabbit chromaffin cells of 18-day old fetuses (COUPLAND and WEAKLEY, 1968) and 16- to 18-day rat fetuses (ELFVIN, 1967; ELMAGHRABY and LEVER, 1980).

Recent work (VERHOFSTAD et al., 1985) has confirmed and extended observations (TEITELMAN et al., 1979) on the appearance of catecholamine-synthesising enzymes in the rat and shown the presence of NA, A, DBH and PNMT in all developing chromaffin cells from foetal day-18 to the second to third postnatal day.

Prior to foetal day-18 chromaffin cells were immunoreactive for only NA and DBH. After the 2nd to 3rd postnatal day, A storing cells were immunoreactive for NA (moderate) and for A (intensely) and for DBH and PNMT. Typical NA cell islands within the adrenal medulla were not observed prior to the 2nd to 3rd postnatal day and reacted negatively for both A and PNMT.

These latter findings have been correlated recently with the ultrastructural identification of A- and NA-storing chromaffin cells postnatally (TOMLINSON and COUPLAND, unpublished). At day-0, 56% of cell profiles contain predominantly A-type granules while 38% contain predominantly NA-type granules. By day-4 *c* 17% of cells contain only or mainly NA granules while the remainder are unequivocally A cells. By day-8 all chromaffin cells are unequivocally A or NA storing except for the very occasional small granule chromaffin (SGC) cell.

The common origin of chromaffin cells and post-ganglionic sympathetic neurons is now undisputed and their potential plasticity *in vivo* during foetal life is evident from the effects of injections of nerve growth factor (NGF; LEVI-MONTALCINI and ALOE, 1980) which result in an adrenal medulla composed of cells having the characteristics of sympathetic neurons. Under normal *in vivo* conditions the proximity

of the notochord and the sclerotomal part of the somite to the migrating neural crest cells are vital for their development of adrenergic features (LE DOUARIN, 1980).

Corticosteroids

Another factor, reported by SHEPHARD and WEST (1951) and COUPLAND (1953) as being of possible importance in determining the nature of the catecholamine stored by chromaffin cells, is the adrenal cortex. The mechanism became clear when WURTMAN and AXELROD (1966) reported that in post-natal animals the induction of PNMT was dependent upon the presence of 11-oxycorticosteroids. COUPLAND and MACDOUGALL (1966) and COUPLAND (1968) showed that *in vitro* extra-adrenal NA-storing chromaffin cells could be transformed into A-storing cells by corticosterone but not be 11-deoxycorticosterone. Later, however, TEITELMAN et al. (1979) noted that administration of dexamethasone to pregnant rats from 12–16 days of gestation failed to accelerate the appearance of PNMT in the adrenal medulla and suggested that during foetal life the initial appearance of PNMT depends on local environmental factors.

The many different trophic factors now known to influence the survival, differentiation and phenotype of adrenal medullary cells *in vitro* have been reviewed and discussed by UNSICKER (1982) and GROTHE et al. (1985). DOUPE et al. (1985) published a comprehensive study on the effects of NGF, glucocorticoids and non-neuronal cell conditioned media on the survival and biochemical and morphological characteristics of adrenal chromaffin cells in dissociated cell cultures. This has clarified many early reports concerning cell loss, affecting in particular A cells, and the development of adrenergic or cholinergic properties by chromaffin cell-derived neurons. They showed that good survival of cells with a typical chromaffin cell phenotype and normal proportions of A and NA was dependent on the presence of low concentrations of glucocorticoids. They confirmed previous observations that corticosteroids and NGF appeared to act in competition on NGF-induced neurite outgrowth. In the absence of corticosteroids typical chromaffin cells died or transformed into chromaffin cell phenotypes that were predominantly NA-storing and which tended to extend neurites. In the presence of NGF and the absence of glucocorticoids conversion to typical adrenergic neuronal phenotype occurred; in the presence of heart cell conditioned medium these became cholinergic neurons.

In addition to their role in the differentiation of adrenal medullary chromaffin cells glucocorticoids have a specific effect on the growth and survival of extra-adrenal chromaffin tissue in the rat and mouse (LEMPINEN, 1964; MONKHOUSE and COUPLAND, 1985).

Recently observations have been made of the effects of hydrocortisone on the volume of chromaffin tissue and on the labelling index and fraction of labelled mitoses in chromaffin cells *in vivo* in the perinatal and adult mouse (MONKHOUSE and COUPLAND, 1985; MONKHOUSE, 1986). A highly significant increase in the volume (hyperplasia) and labelling index of extra-adrenal chromaffin tissue in mice of 16 days gestation was noted after steroid administration (from day-8 to-16), with only a slight increase in the labelling index of intra-adrenal chromaffin cells. Similar effects were noted after hydrocortisone administration during the last week of gestation and during the first post-natal week. Following the cessation of glucocorticoid administration to postnatal animals the main extra-adrenal chromaffin body disintegrated and only scattered retroperitoneal chromaffin cells remained. Hydrocortisone had no effect on either adrenal medullary volume or labelling index in the postnatal animal. Labelling was significantly higher in A cells than NA cells at 21 days.

Hydrocortisone administered to newborn rats also causes hyperplasia of small intensely fluorescent (SIF) cells in sympathetic ganglia (ERÄNKÖ and ERÄNKÖ, 1972). To date it is not known whether all the SIF cells or only the type I or type II cells (see CHIBA and WILLIAMS, 1975) respond.

In adult animals maximum achievable reductions in endogenous corticosteroids (following hypophysectomy and metyrapone administration) have no effect on either adrenal medullary volume or the proportions of A and NA cells in the rat (COUPLAND et al., 1984). Hence it would appear that *in vivo* once the normal adult A or NA phenotype is achieved it persists. Furthermore after unilateral adrenalectomy in the neonate or adult the remaining adrenal medulla shows no evidence of compensatory growth (TOMLINSON et al., 1987a).

Quantitative morphology of the rat adrenal medulla and of chromaffin cells

Until recently the detailed descriptions of the ultrastructure of the adrenal medulla have lacked quantitative parameters. Yet the chromaffin cell is now commonly used as a model for the sympathetic neuron, in studies on the cell and molecular biology of

exocytosis and on the mechanisms of synthesis, storage and release of catecholamines, proteins and neuropeptides.

Many of these studies would be facilitated if an accurate quantitative baseline of normality at different ages were available. Some five years ago my colleagues and I embarked upon this project and our initial results relating to young adult Wistar rats have already been published (TOMLINSON et al., 1987b).

A striking finding to date has been the wide normal variation in relative numbers of A and NA cells and amine concentrations between litter mates and animals of the same inbred colony identical in weight and of similar age. A similar variation has also been reported in Sprague-Dawley rats (COUPLAND et al., 1984). Thus NA cells may form 13-28% of adrenal chromaffin tissue in adult Wistar and 16-38% in Sprague-Dawley rats, with similar variations in NA concentration. In some 90% of animals the left adrenal medulla is larger than the right and has a higher amine content. These variations highlight the importance of reproducible techniques and adequate numbers in any experimental procedures.

Recent results (and TOMLINSON and COUPLAND, unpublished) have confirmed that both A and NA cells contain a full range of cytoplasmic organelles and apart from the well accepted differences in the appearance and electron density of A and NA granules the only subcellular component showing a significant difference between A and NA cells is rough endoplasmic reticulum which in the young adult animal has a three times greater volume density (V_v) in A cells.

The volume of the adrenal medulla was observed to increase throughout adult life. In 250 gm Wistar rats medullary tissue components included chromaffin cells 63%, vascular 20%, neuronal 5% and interstitial tissues 12%. The average numbers of chromaffin cells in the adrenal medullas examined ranged from 4.4×10^5 to 5.7×10^5 A cells and 1.5×10^5 to 1.9×10^5 NA cells. The mean volume of an A cell was $1300 \mu\text{m}^3$, and of NA cell $980 \mu\text{m}^3$. The mean diameter of an A granule increases from 184 nm at birth to 227 nm at 17 weeks.

Assuming all A and NA is stored in the specific cell type (unlikely), the mean amine content of A and NA cells would be $0.14 \times 10^{-6} \mu\text{moles}$ and $0.17 \times 10^{-6} \mu\text{moles}$ respectively and that of an A granule 3.2 or $3.8 \times 10^{-12} \mu\text{moles}$ and an NA granule $5 \times 10^{-12} \mu\text{moles}$. The number of chromaffin granules in an A cell was 37,000 or 47,500, (the two figures given relate to calculations using N_v or V_v as the basis). Based on N_v determination a NA cell contains 34,000 granules.

SMALL-INTENSELY FLUORESCENT (SIF) AND CHROMAFFIN CELLS

The presence of chromaffin cells in sympathetic ganglia has been reported for many years (see COUPLAND 1965a, 1978). Although species variable and often sparsely distributed they occur in both pre- and paravertebral sympathetic ganglia and persist throughout adult life-unlike some of the prevertebral extra-adrenal chromaffin bodies.

Using the formaldehyde-induced fluorescent technique for catecholamines, ERÄNKÖ and HARKONEN (1963) noted small intensely fluorescing (SIF) cells as well as sympathetic neurons in the superior cervical ganglion of the rat—a species in which SIF cells are particularly abundant (CHIBA and WILLIAMS, 1975). Later, granule-containing cells were observed in the rat superior cervical ganglion by electron microscopy (SIGRIST et al., 1966; GRILLO, 1966). WILLIAMS (1967) described an efferent synapse between a cell containing small dense-cored vesicles and an adjacent sympathetic neuron and suggested that the small cell may function as an interneuron. Later MATTHEWS and RAISMAN (1969) described both afferent and efferent synapses associated with SIF cells, thus supporting the interneuron concept.

CHIBA and WILLIAMS (1975) suggested that SIF cells could be subdivided into two types, Type I having processes and possibly being interneurons and Type II being more compact, having larger granules and often being closely related to blood vessels. The first attempt to correlate the occurrence of SIF cells with that of chromaffin cells in sympathetic ganglia appears to be that of SANTER et al. (1975). SIF type I cells were the main fluorescent cell type in the rat superior cervical ganglion and chromaffin cells (which would resemble type II SIF cells) are few or absent; chromaffin cells were more frequently observed in thoracic and abdominal ganglia. No convincing evidence for the occurrence of interneuronal SIF cells (type I) in human sympathetic ganglia has been presented (HERVONEN and KANERVA, 1972; CHIBA, 1978; HERVONEN et al., 1979) and the cells illustrated in these publications were typical extra-adrenal chromaffin cells in spite of the SIF designation.

Superficially at least, the SIF type I cells resemble both chromaffin cells in culture in the presence of nerve growth factor or less than optimal concentrations of glucocorticoids, and in some respects the SGC cells of the mouse adrenal medulla (KOBAYASHI and COUPLAND, 1977; KOBAYASHI et al., 1978).

DOUPE et al. (1985) described the differentiation of

SIF cells *in vitro* into either neuronal or chromaffin cell phenotypes, according to the concentrations of growth factors and glucocorticoids in culture. They also suggested that SIF cells may form the intermediate developmental stage between primitive sympathoadrenal cells and both chromaffin cells and autonomic neurons. The only adrenal medullary cell which may be a contender for such a designation is the SGC cell.

In my experience SGC cells are rarely observed in the adrenal medulla in mammals other than the mouse in which they form 4–5% of chromaffin cells (COUPLAND, 1984). In the latter situation they are almost certainly not specific DA-storing cells and, as suggested by the work GORGAS and BÖCK (1976), may be an unusual form of NA cell; they respond to insulin hypoglycaemia in the same way as the latter (COUPLAND et al., 1978). The electron microscopic appearance of the chromaffin granules of the SGC cells would be in keeping with mixed (A and NA) storage.

IF SIF (SGC) cells are precursors of chromaffin cells it would be likely that during the first two weeks of postnatal life these cells in the mouse would be the ones to undergo mitosis or take up ^3H -thymidine. Yet there is no evidence of a peripheral distribution of labelled cells during the period of rapid adrenal medullary growth or a peripheral location of mitotic figures (MONKHOUSE, unpublished). Furthermore mitotic figures are always observed in chromaffin cells with the typical granule appearance of the definitive cell type, usually those of mature A cells.

It is apparent that the type I SIF cell is morphologically similar in many respects to the elements referred to throughout the past century as pheochromoblasts. Furthermore, there is currently no direct evidence from *in vivo* studies that the primitive sympathetic cell differentiates into an SIF type I phenotype before differentiating into chromaffin cells and neuroblasts. Once committed to an endocrine phenotype the initial stage, the pheochromoblast, does however bear a superficial morphological resemblance to the SIF type I cell of the rat sympathetic ganglion. Furthermore, the attenuated form of SGC cell not infrequently seen in the mouse and which has a superficial resemblance to a SIF type I cell is virtually never seen in other forms viz, rat, guinea pig, rabbit (COUPLAND, unpublished) dog (KAJIHARA et al., 1978), or cat (AUTILLO-TOUATI, 1979) and SIF type II cells are indistinguishable from chromaffin cells.

One must therefore question whether the use of the term SIF cell for other than the type I cell with

processes is justified and whether its extension into established embryological terminology will serve a useful purpose. Or will confuse? As did attempts based on histological criteria to introduce the concept of the occurrence of chromaffin cells in the skin and gut in ungulates that were subsequently shown to be mast cells (COUPLAND and HEATH, 1961a, b).

Innervation

Recent work has demonstrated that the functional innervation of the adrenal medulla in the rat is relatively late to mature (see SLOTKIN, 1986).

At the same time LIVETT et al. (1979), BOSKA and LIVETT (1984) and KHALIL et al. (1986) have implicated substance P, in the response of chromaffin cells to nicotinic stimulation both *in vivo* and *in vitro*.

These reports have prompted an extension of our quantitative investigations on the functional morphology of the adrenal medulla into the sensory and motor innervation of the rat and guinea pig adrenal medulla. In relation to this work I would like to highlight particularly the contribution of my colleague, Dr Terry PARKER. Quantitative studies have followed nerve fibre tracing after the injection of 3–5 μl Fast Blue or horse-radish peroxidase into the adrenal medulla, great care being taken to avoid contamination of either the surface of the gland or the peritoneal cavity and to deliver the tracer at or near the centre of the adrenal medulla.

Pre- and post-ganglionic sympathetic innervation of adrenal medulla

The recent work (KESSE et al., 1988) has confirmed the presence of preganglionic cell bodies in the ipsilateral intermediolateral horn of the spinal cord of the Wistar rat between segments T1 and L1. On the left side the greatest numbers are found in segments T9–10. The mean number of preganglionic neurons was 698 ± 27 (S. E. M.). Labelled postganglionic cell bodies were found in paravertebral sympathetic ganglia at levels T4–T12 total 68 ± 2.5 (S.E.M.), with the maximum numbers at T9 and T10; in addition labelled cells were found in the ipsilateral suprarenal ganglion (21 ± 1.7) but not in other prevertebral ganglia. Hence the mean total of postganglionic neurons was 89.

In the guinea pig, using the same techniques (PARKER, MOHAMED and COUPLAND, unpublished) labelled preganglionic cell bodies were observed in the ipsilateral intermediolateral horn of the spinal cord from T3 to L2, with maximum numbers on the left side, in segments T8–10 and a mean total of 553 ± 15 .

Labelled postganglionic sympathetic neurons were seen in the ipsilateral paravertebral ganglia at levels T3 to T12 with the majority at T9–12. As in the rat the suprarenal ganglion was the only prevertebral ganglion containing labelled neurons. Mean total of postganglionic neurons was 164.

Spinal afferent innervation

Labelled neurons were observed in ipsilateral dorsal root ganglia of rat and guinea pig, from T3–L2 on the left side (MOHAMED et al., 1988) (PARKER, AFEWORK and COUPLAND, unpublished) with the majority at T10; mean numbers were rat 164, guinea pig 503. In the rat after neonatal treatment with capsaicin there was *c* 40% reduction in the number of labelled neurons though the distribution pattern remained the same. The neurons lost after capsaicin belonged to the small diameter fibre group.

Vagal innervation

A vagal innervation was present in both rat and guinea pig with visceral efferent cell bodies situated in the dorsal motor nucleus of the vagus bilaterally (122 in guinea pig, 20 in rat) and visceral afferent cell bodies present bilaterally in the vagal sensory ganglia, especially nodose (50 in guinea pig, 26 in rat). In both groups there was a slight ipsilateral predominance in the innervation (COUPLAND et al., 1989). Following neonatal capsaicin treatment in the rat there was a 55–61% loss of neurons in the ipsi- and contralateral vagal sensory ganglia respectively (PARKER, AFEWORK and COUPLAND, unpublished).

These findings imply a ratio of preganglionic sympathetic neurons to chromaffin cells of 1 : 1000 in the rat.

The preganglionic sympathetic innervation of the guinea-pig adrenal gland is provided by *c* 550 neurons (553.2 ± 15.2 SEM, *n*=10). The sensory innervation involves some 500 spinal sensory neurons, and in addition some 50 vagal sensory ganglion cells were labelled. The parasympathetic efferent innervation to the adrenal gland is considerably greater in the guinea pig than in the rat and after the injection of Fast Blue into the left adrenal gland some 120 labelled neurons were observed within the ipsi- and contralateral dorsal motor nuclei of the vagi.

At the present time the peripheral termination of vagal sensory and efferent fibres within the adrenal gland is unknown though, because of the injection technique many are likely to be within the adrenal medulla: profiles of possible sensory endings have been observed. The peripheral termination of vagal efferent fibres has to be determined.

Once the location of specific nerve endings has been identified and their neuropeptide characteristics determined, we shall be in a better position to answer the questions raised by Livett and co-workers on the role of substance P and sensory neurons during cholinergic stimulation of adrenal medullary secretion. In the meantime the very significant and widely distributed sensory input from the adrenal gland to the spinal cord and brain stem suggests the involvement of these fibres in both segmental reflex activity and more general homeostatic mechanisms.

Neuropeptides

The contents of a review of this nature are inevitable limited by time and space. Consequently only brief reference will be made to neuropeptides.

In view of their abundance and likely importance enkephalins and opioid-like substances must first be mentioned. Enkephalin-like immunoreactivity was demonstrated in both nerve terminals and chromaffin cells in guinea pigs in 1978 (SCHULTZBERG et al., 1978) and VIVEROS et al. (1979) demonstrated the simultaneous release of enkephalins and catecholamines from the isolated perfused dog adrenal gland, thus raising the possibility that it may have a hormonal function. This possibility has also been considered with respect to chromogranins for more than 20 years and recently it has been suggested that chromogranin A may be a prohormone precursor for pancreastatin (EIDEN, 1987).

Recent reports on neuropeptides have demonstrated species variability, differences in expression *in vivo* and *in vitro* and depending upon innervation and applied stimuli. Recent work in this department (KENT and COUPLAND, unpublished) has demonstrated changes in the localization of enkephalin-like immunoreactivity shortly after birth that can be correlated with and indeed reflect functional innervation.

In addition to the ubiquitous neuropeptides associated with chromaffin cells and the peripheral autonomic nervous system such as enkephalins, vasoactive intestinal peptide, substance P, neuropeptide Y, calcitonin gene-related peptide, somatostatin and neurotensin, atrial natriuretic peptide- (DELEAN et al., 1985; PRUSS and ZAMIR, 1987) oxytocin- and vasopressin- (ANG and JENKINS, 1984; HAWTHORNE et al., 1987) and corticotropin-releasing-like immunoreactivity (HASHIMOTO et al., 1984) have been reported in chromaffin cells in a variety of mammalian species.

Although many of the neuropeptides and hormones associated with chromaffin cells and/or their innerva

tion are likely to be involved in fine tuning of homeostatic mechanisms and sympathoadrenal activity including responses to stress, it is apparent that we are still some distance away from a full understanding of their functional role.

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