

The innervation of the mammalian adrenal gland

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ABSTRACT

Early conflicting reports and the lack of sensitive anatomical methods have led to an oversimplified view of adrenal gland innervation. It was not until the introduction of nerve fibre tracing techniques in the mid-1970s that the true complexity of adrenal innervation began to emerge. The first part of this article comprises a brief review of these and other relevant reports dealing with both medullary and cortical innervation. In the second part a detailed account is given of the work undertaken in Rex Coupland's Department relating to the innervation of the rodent and primate adrenal medulla using a retrograde fluorescent tracer technique. It was concluded that, in all 3 species studied, the adrenal medulla receives a sympathetic and parasympathetic efferent and an afferent innervation. The possible interrelationship between neural control of cortical and medullary secretions is discussed briefly.

INTRODUCTION

The development of new techniques in recent years for tracing nerve fibre connections has stimulated an extensive reexamination of the traditional views on the innervation of the adrenal gland. In his classic book *The Natural History of the Chromaffin Cell*, Coupland (1965) reviewed the early literature concerning adrenal gland innervation up to that date. It revealed that the innervation of the adrenal had been the focus of extensive research spanning almost a century. Although these early studies were based either on gross dissection or on the relatively insensitive cell degeneration technique, many of the conclusions have subsequently proved to be correct. Evidence was presented for a preganglionic (Elliot, 1913; Hollingshead, 1936; Young, 1939) and post-ganglionic sympathetic (Hoshi, 1926; Swinyard, 1937) and parasympathetic (Teitlebaum, 1933) innervation, together with indirect evidence for a sensory afferent component (Pines & Narowtschatowa, 1931). However, despite this compelling evidence, there has been a reluctance to incorporate this knowledge into general textbooks, and most chapters on the autonomic nervous system refer only to the preganglionic sympathetic component of adrenal innervation. The reference to other modes of medullary innervation

and the possibility of nervous control of cortical cells is mostly confined to the research literature. During the intervening years since 1965, evidence has steadily accumulated to support the earlier studies. In the first part of this article the accumulated knowledge regarding adrenal gland innervation will be reviewed briefly; in the second part we will present our views specifically on the innervation of the adrenal medulla as revealed by retrograde tracer studies undertaken in this Department over the past 5 years.

General aspects of adrenal gland innervation

The cortex and medulla of the adrenal gland traditionally have been regarded as independent entities, and as such have been studied separately by either endocrinologists or neuroscientists. This has led to a divorced approach to the understanding of the role played by the vasculature and innervation in adrenal function. More recent studies on adrenal vasculature have attempted to integrate the blood supply to the 2 regions. The cortical and medullary arteries have been shown to have separate external origins and form distinct capillary beds in the 2 regions (Coupland, 1975; Coupland & Selby, 1976; Kikuta & Murakami, 1982, 1984). Importantly, the lack of a portal system linking the cortex and medulla was confirmed by

Sparrow & Coupland (1987), who found that 7.4% of the total adrenal blood flow passes directly to the medulla, the remainder passing to the cortex. The nature of the capillary network within the gland may have an important bearing on the distribution of nerve fibres to the gland and will be considered in more detail later. With regard to adrenal innervation, this has traditionally been concerned with the medulla, and it is only recently that the cortex has even been considered to be innervated. It is not surprising therefore that there is little or no evidence to suggest that the source of innervation to the cortex and medulla are separate and that both regions share a wide range of extensively branched fibres carrying both efferent and afferent modalities differing only in quantitative distribution. Similarly, there is no evidence to disprove the notion that fibres projecting to the cortex or medulla originate in different parts of the CNS. Indeed, both respond to stress and both are controlled, at least in part, by the hypothalamus.

However, it is generally accepted that the cells of the adrenal gland receive both an intrinsic and an extrinsic innervation. The majority of external fibres projecting to the adrenal travel via the splanchnic nerves. After penetrating the capsule they branch to form an extensive subcapsular network; from here fibres pass to both cortex and medulla to form further networks surrounding the cells of these regions. The intrinsic innervation arises from ganglion cells sparsely distributed throughout the gland in subcapsular, cortical and medullary regions (Coupland, 1965; Lewis & Shute, 1969). These points will now be considered in more detail.

Extrinsic innervation

Adrenal medulla. Classical cholinesterase and nerve degeneration studies have demonstrated that the majority of the fibres projecting to the medulla are cholinergic preganglionic sympathetic fibres arising predominantly from the cord at spinal levels T7-T9 (Hoshi, 1926; Hollinshead, 1937). The advent of modern retrograde fibre tracing techniques produced several reports essentially verifying these earlier studies (Ellison & Clark, 1975; Schramm et al. 1975; Haase et al. 1982; Holets and Elde, 1982). The early suspicions of Hoshi (1926) and Swinyard (1937) that the adrenal received a postganglionic innervation were supported by the later physiological studies of Celler & Schramm (1981). Similarly, the earlier evidence for an afferent supply to the adrenal gland by Pines & Narowschatowa (1931) and Kiss (1951) were supported by the physiological evidence of Nijima &

Winter (1968). Then in the late 1980s Coupland and his coworkers provided unequivocal morphological evidence for both a preganglionic and postganglionic sympathetic and parasympathetic and an afferent innervation to the rat and guinea pig adrenal (Afework, 1988; Kesse et al. 1988; Mohamed et al. 1988; Coupland et al. 1989; Parker et al. 1990*b*). These studies provided quantitative information on the source and contribution of the different projections to the adrenal medulla. The most recent reports show that postganglionic fibres to the adrenal gland, in part, innervate cortical blood vessels (Carlson et al. 1990). The afferent cell bodies in both the dorsal root ganglion and vagal sensory ganglia projecting to the adrenal medulla were shown to be capsaicin sensitive (Parker et al. 1990*a*) and that these are substance P-containing fibres (Zhou et al. 1991).

Adrenal cortex. Conventionally, steroid secretion from the cortex is thought to occur in response to hormonal stimuli: adrenocorticotrophic hormone (ACTH), androgens, oestrogens and angiotensin. But as pointed out by Edwards (1990), at least as far as ACTH is concerned, there is often a marked discrepancy between changes in adrenal glucocorticoids and plasma ACTH levels. This points to some additional mechanism for the control of steroid secretion. There is growing evidence that neural activity can directly affect endocrine organs, and as is true for the adrenal medulla, several early studies showed that the adrenal cortex contains nerve fibres (Dogiel, 1894; Alpert, 1931; Willard, 1936). However, these fibres were thought merely to pass through the cortex prior to innervating chromaffin cells of the medulla. It was not until ultrastructural studies provided evidence for terminals containing synaptic vesicles adjacent to cortical cells that the case for nervous control of steroid secretion gained momentum (Unsicker, 1971; Garcia-Alvarez, 1972; Robinson et al. 1977). Since this time, substantial support has accumulated from physiological and biochemical studies both in vivo and in vitro for cortical innervation (for extensive reviews, see Unsicker, 1984, Holtzworth et al. 1987, Charlton, 1990, and Edwards, 1990). However, although it seems likely that the nervous system is involved in the control of cortical secretion, evidence is still lacking as to the exact nature of the circuitry, in particular the source of nerve fibres projecting to the adrenal cortex. In this article it is not intended to present an exhaustive account of cortical innervation; however, it is felt that the innervation of the cortex and medulla should be considered together and as possibly sharing nervous control either directly or indirectly via some form of integrated neural/

humoral or vascular circuit (see e.g. Bornstein et al. 1990; Hinson, 1990).

Intrinsic innervation

The intrinsic innervation has received less attention since the first reports of bipolar or multipolar nerve cells in the adrenal medulla (Dogiel, 1894). It consists of ganglion cells located either under the capsule or scattered between cortical and medullary cells (Coup-land & Holmes 1958; Coup-land 1965; Shioda & Hishida, 1967; Lewis & Shute, 1969; Unsicker, 1971; Unsicker et al. 1978; Migally, 1979; Kleitman & Holzwarth, 1985; Watanabe et al. 1990). Dagerlind and Hökfelt (1991) showed that at least some of the intrinsic ganglion cells located in the medulla project to the cortex and Oomori et al. (1991) showed that some of the fibres are in close apposition to pericytes of blood vessels in the cortex and chromaffin cells in the medulla. The number of ganglion cells is species dependent and they are considered to arise from sympathetic neurons that have differentiated from neural crest cells which have migrated into the cortical anlage during development. These ganglion cells, in addition to being AChE positive, also contain met- and leu-enkephalin, NPY and VIP immunoreactive neuropeptides (Pelto-Huikko et al. 1985; C. H. Heym & M. Colombo-Benkmann, unpublished observations; A. Dagerlind & T. Hökfelt, unpublished observations). There is some evidence from Le Douarin's work (1982) to suppose that, at least in the avian adrenal gland, some of these ganglion cells may arise from vagal regions of the neural crest. These ganglion cells may therefore be both the source of sympathetic and parasympathetic intrinsic postganglionic fibres within the gland as well as providing the destination for at least some of the extrinsic fibres projecting to the gland.

Distribution of axon terminals and fibres within the adrenal gland

There is still some confusion as to the distribution of the different types of axon terminals found in the adrenal gland. Evidence continues to accumulate regarding the types of peptide-containing terminals: enkephalins (Schultzberg et al. 1978; Kobayashi et al. 1983, 1985; Hexum & Barron. 1984; Pelto-Huikko et al. 1985); VIP (Hökfelt et al. 1981; Holzwarth, 1984); NPY (Varndell et al. 1984); substance P (Pfister & Gorne, 1983; Gorne et al. 1984; Livett, 1987; Livett et al. 1990). With regard to the classical autonomic terminals, cholinergic terminals are found throughout

the cortex and medulla and are still regarded as the main type of innervation. Adrenergic terminals are found in the cortex in association with cortical cells and blood vessels (Unsicker, 1971; Carlson et al. 1990). However, the distribution of these terminals in the medulla remains uncertain with only 2 reports of adrenergic terminals innervating chromaffin cells (Piezzi, 1966; Prentice & Wood, 1975).

Receptors

Catecholamine secretion from the adrenal medulla is mediated by nicotinic cholinergic receptors. While there is little doubt that this is the prime mechanism for catecholamine release, evidence is accumulating to show that other receptors may be involved in modulating this response. In addition, it is well known that muscarinic receptors are also present on chromaffin cells (Feldberg et al. 1934) and that these may be involved in catecholamine release (Douglas & Poisner, 1965; Role & Perlman, 1983; Wakade & Wakade, 1983; Schneider, 1987; Jones & Edwards, 1991). The distribution of the 2 subtypes of cholinergic receptor is markedly species-dependent, and that they appear to behave differently under *in vivo* or *in vitro* conditions (Malmejac, 1964; Rubin & Miele, 1968; Liang & Perlman, 1979; Knight & Baker, 1986). The discovery of a variety of neuropeptide receptors on chromaffin cells has opened up a new era in the understanding of the role of the classical transmitter/receptor in catecholamine secretion. Some neuropeptides are now thought to be directly involved in the release of catecholamines, for example angiotensin II (Marley et al. 1989), while substance P, for example, has been shown to play a modulating role in catecholamine secretion (Zhou & Livett, 1990; for extensive reviews, see Marley & Livett, 1985, and Livett, 1987).

Although there is less morphological information regarding the distribution of cortical receptors, it would seem from pharmacological studies that a similar pattern of cholinergic and peptidergic receptors are present on cortical cells, lending further support to the concept of cortical innervation.

Central connections

The brainstem and hypothalamus are the 2 regions thought to control sympathoadrenal outflow (Holets & Elder, 1982; Robinson et al. 1983; Stoddard-Apter et al. 1983; Matsui, 1984; Goadsby, 1985). The most recent studies of Strack et al. (1988, 1989) and Wesselingh & Blessing (1989) using transneuronal cell

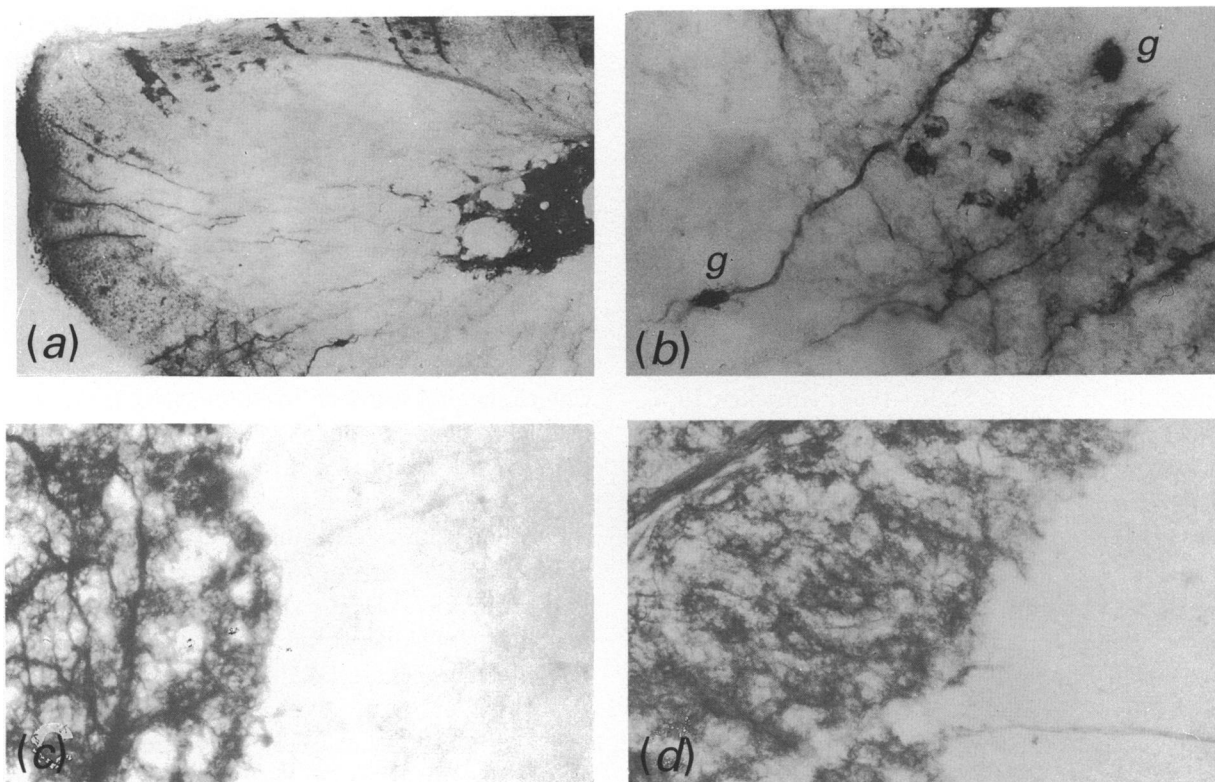


Fig. 1. AChE-stained nerve fibres and ganglion cells in the adrenal glands of guinea pig (*a, b*), rat (*c*) and marmoset (*d*). Note the heavy staining of fibres in the medulla and relatively sparse staining in the cortex. The AChE staining fibres can be clearly seen traversing the cortex in the guinea pig (*a*); note also the heavily stained ganglion cells (*g*). (*a*, $\times 80$; *b, c, d $\times 300$).*

body labelling with pseudorabies virus found 5 regions within the brainstem and hypothalamus controlling intermediolateral neurons, and Bacon & Smith (1988) identified 4 distinct types of afferent synaptic input to these neurons. Following the demonstration by Folkow & von Euler (1954) of dual control of catecholamine release in the cat, several authors have shown that stimulation of specific sites in the CNS can lead to preferential secretion of adrenaline and noradrenaline from the adrenal medulla (Robinson et al. 1983; Stoddard-Apter et al. 1983; Matsui, 1984; Kataguchi et al. 1986). Interestingly, Edwards (1982) has produced a similar effect by varying the pattern of splanchnic nerve stimulation in the conscious calf (for references, see Edwards, 1990).

METHODS

Since the mid-1980s we have been studying the source of nerve fibres innervating the adrenal medulla of rat, guinea pig and marmoset using the retrograde fluorescent tracer fast blue. This tracer was extensively developed by Kuypers & Huisman (1984) and we found it excellent for use in the peripheral nervous system. A full account of the method of tracer injection and the extensive control experiments can be

found in Kesse et al. (1988) and Kesse (1988). Briefly, 5 μ l of a 2% aqueous suspension of fast blue was pressure-injected unilaterally into either the right or left adrenal medulla under neuroleptic anaesthesia (Hypnorm, Janssen, 1 ml/kg – phentanyl citrate 0.31 mg and fluanisone 10 mg/ml, and diazepam 2.5 mg/kg). After a period of 5 d the animals were perfused transcardially under barbiturate anaesthesia (sodium pentobarbitone anaesthesia 60 mg/kg). The tissues for examination were removed and serial cryostat sections prepared prior to examination in a Leitz microscope fitted with the Ploempack fluorescent system and excitation wavelength of 390 nm. AChE histochemistry was performed according to the method of Karnovsky & Roots (1964) and using the pseudocholinesterase inhibitor tetraisopropylpyrophosphoramidate (iso-OMPA).

Catecholamines were analysed according to the method described in Verhofstadt et al. (1985).

RESULTS

AChE histochemistry

The pattern of cholinergic fibres in the adrenal gland varied appreciably between the 3 species (Fig. 1). The most extensive network was seen in the guinea pig

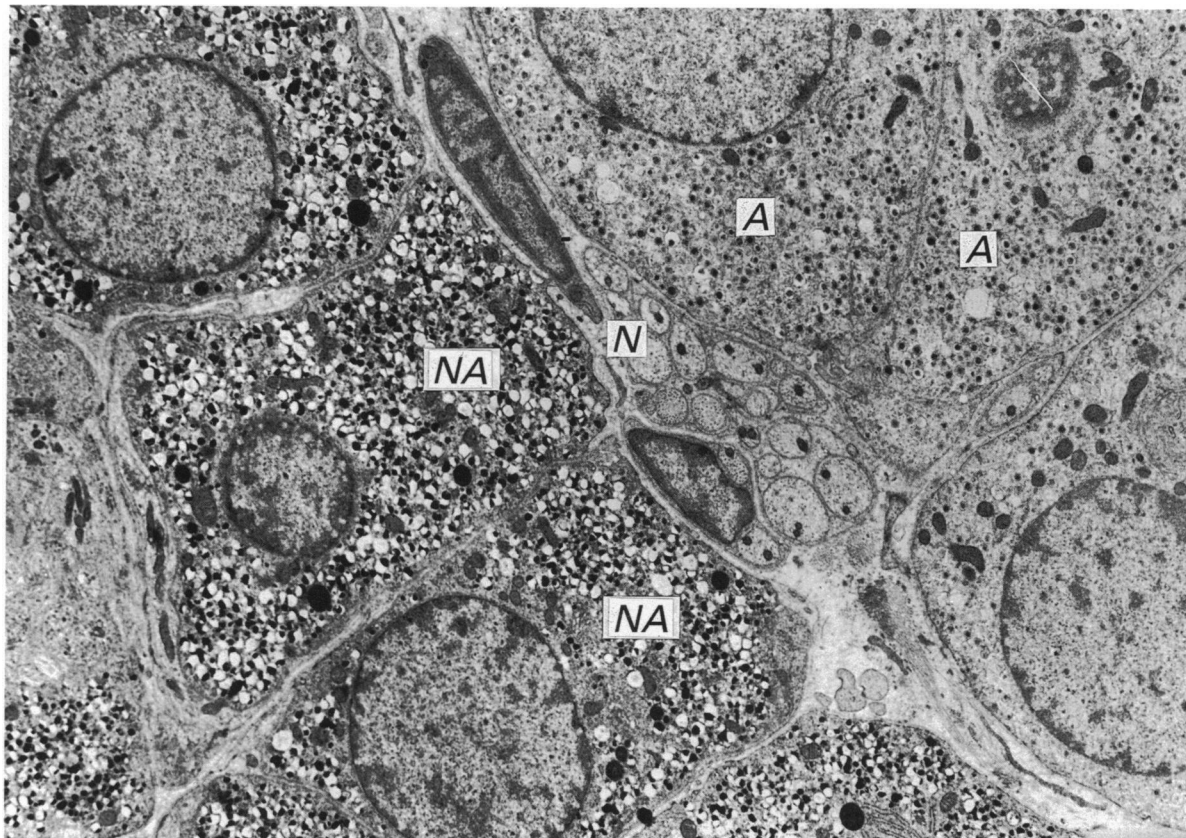


Fig. 2. Ultrastructure of the rat adrenal medulla. Note the 2 types of storage granules (*NA* and *A*). Unmyelinated nerve fibres (*N*) are present, passing between the chromaffin cells.

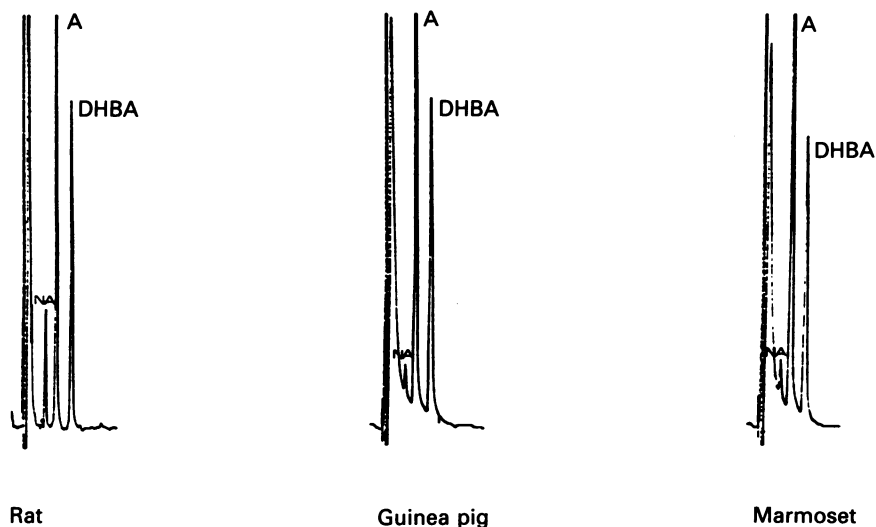


Fig. 3. HPLC chromatograms of adrenal extracts of rat, guinea pig and marmoset. Note the peaks of adrenaline (*A*) and noradrenaline (*NA*). Both the marmoset and guinea pig have small amounts of noradrenaline as compared with the rat. DHBA, 3,4-dihydroxybenzylamine standard. Rat: *A*, 116.6 ± 2.6 nmol/gland; *NA*, 29.0 ± 4.5 nmol/gland. Guinea pig: *A*, 147.8 ± 13.2 nmol/gland; *NA*, 5.8 ± 1.2 nmol/gland. Marmoset: *A*, 122.2 ± 5.7 nmol/gland; *NA*, 6.21.1 nmol/gland.

with a well developed subcapsular arrangement giving off fibres that either branched in the cortex or appeared to pass directly to the medulla (Fig. 1*a*). The medulla contained the most extensive network of

fibres. Many of the fibres formed branches which either ran between the cortical cells or contacted AChE-positive neurons (Fig. 1*b*). In the rat adrenal gland, most of the AChE-positive fibres were concen-

trated in the medulla forming an extensive network in which were embedded small strongly staining ganglion cells (Fig. 1c). The cortex, in contrast, contained a few lightly staining fibres with no apparent branching. The pattern of AChE-positive fibres in the marmoset adrenal appeared to be some way between that of the rat and guinea pig, having an extensive medullary network with a sparse cortical distribution where the fibres appeared to run from the capsule through the cortex, unbranched, to the medullary core (Fig. 1d).

Ultrastructural studies

It is now well established that the rat adrenal medulla is primarily composed of 2 types of chromaffin cell characterised by the type of storage granule contained within the cytoplasm (Fig. 2). The eccentrically located dense core granules contain noradrenaline and the more diffuse spherical granules adrenaline. In contrast, our preliminary morphological data revealed that the adrenal medullas of the guinea pig and marmoset consist almost entirely of adrenaline-storing cells.

Catecholamine analysis

In all 3 species of animal studied only adrenaline and noradrenaline were found in quantifiable amounts. Trace amounts of dopamine were occasionally present in the adrenal extracts. The quantities of stored catecholamine per gland available for release can be seen from Figure 3. The guinea pig and marmoset adrenal chromaffin cells contained almost exclusively adrenaline while the rat chromaffin cells contained approximately 25% noradrenaline. These results support the ultrastructural studies on the distribution of chromaffin cell types within the adrenal medulla of the 3 species.

Sympathetic innervation

Sympathetic fibres innervating the adrenal medulla arose from the spinal cord levels T3-L2, ipsilateral to the site of injection into the left adrenal in all 3 species studied (Fig. 4), the greatest number of fibres arising at T9 and T10. Within these segments the majority of the labelled cells lay within the nucleus intermediolateralis pars principalis with a small proportion of the cells arising in 2 other nuclei, the intermediolateralis pars funicularis and pars intercalatus.

The labelled cells were fusiform ($8 \times 22 \mu\text{m}$) to round ($14 \mu\text{m}$ in diameter). The cell clusters tended to form a beaded arrangement along the cord with many

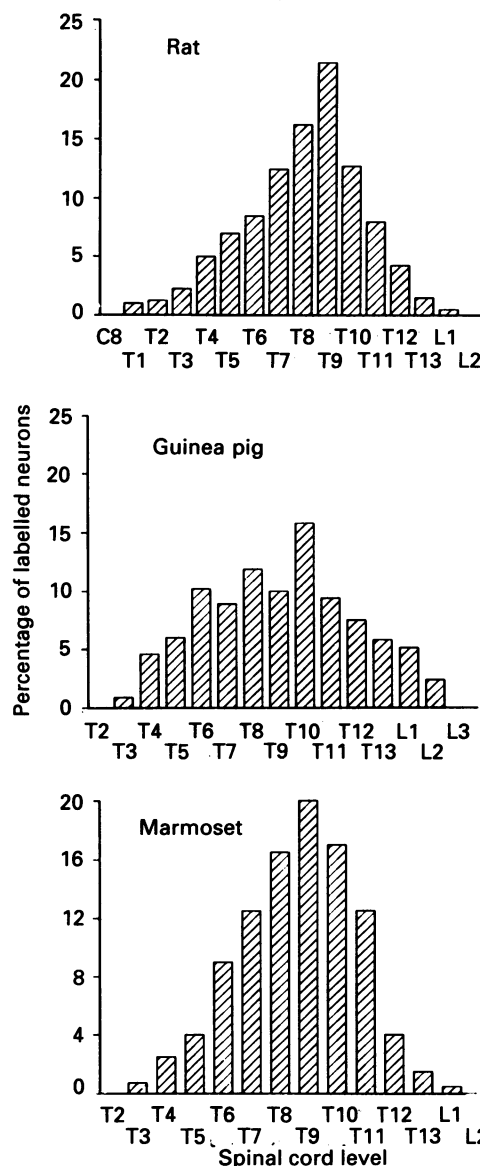


Fig. 4. Percentage contribution of fast blue labelled neurons in the ipsilateral intermediolateral horn of the spinal cord after injection of tracer into the left adrenal gland. Note in rat, guinea pig and marmoset, the major contribution arises from T8 to T11.

dendrites branching near their soma, the longer dendrites often oriented in a mediolateral direction (Fig. 5a). From Table 1, it can be seen that in the rat, guinea pig and marmoset the majority of the labelled cells were found in the spinal cord, indicating that these were preganglionic sympathetic fibres. However, a varying number of neurons projecting ipsilaterally to the medulla synapsed either in the ganglia of the sympathetic chain or in the suprarenal ganglion. These represented a relatively small number of postganglionic sympathetic fibres innervating the adrenal gland (Fig. 5b). The guinea pig received the greatest number of postganglionic fibres in comparison with the 2 other species. The source of the

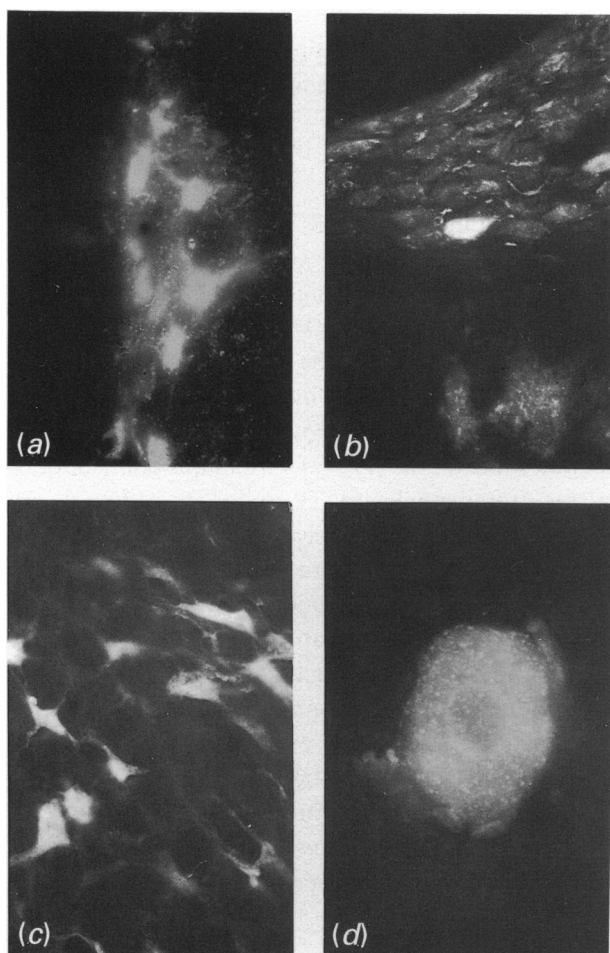


Fig. 5. Fast blue labelled cells nucleus in (a) intermediolateralis pars principalis of the rat spinal cord, $\times 350$, (b) guinea pig sympathetic chain and (c) dorsal motor nucleus of the vagus, $\times 350$ and (d) marmoset dorsal root ganglion, $\times 220$.

Table 1. Sympathetic innervation*

Species	Location	Number (S.E. \pm S.E., n)	Percentage
Rat	Spinal cord	698.4 (S.E. \pm 27.2, n = 10)	88.2%
	Sympathetic chain	68.0 (S.E. \pm 2.5, n = 10)	9.2%
	Suprarenal ganglion	21.0 (S.E. \pm 1.7, n = 10)	2.6%
Guinea pig	Spinal cord	553.4 (S.E. \pm 21.3, n = 8)	77.0%
	Sympathetic chain	125.0 (S.E. \pm 3.4, n = 8)	17.4%
	Suprarenal ganglion	40.0 (S.E. \pm 1.5, n = 8)	5.6%
Marmoset	Spinal cord	362.0 (S.E. \pm 23.4, n = 5)	
	Sympathetic chain	Labelled cells	
	Suprarenal ganglion	Not found	

* The location and number of fast blue labelled cells found in each species expressed as a percentage of the total number of cells labelled per animal.

fibres can be seen from Figure 6. Once again it should be noted that spinal levels T9, T10 and T11 provide the greatest input to the projection. Unfortunately, the labelling in the marmoset was not consistent

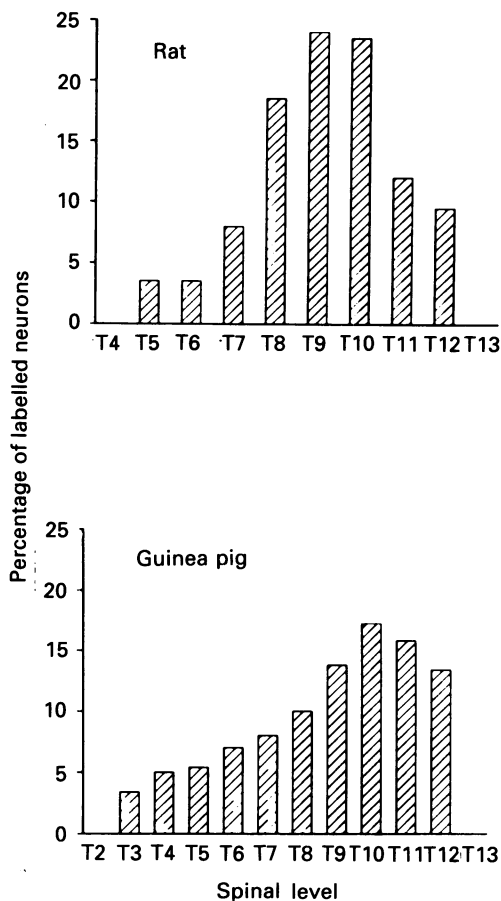


Fig. 6. Percentage contribution of fast blue-labelled neurons in the ipsilateral sympathetic chain after injection of tracer into the left adrenal gland of rat and guinea pig.

Table 2. Parasympathetic innervation*

Species	Location	Number (S.E. \pm S.E., n)	Percentage
Rat	Dorsal motor nucleus of vagus	Inconsistent	
	Guinea pig		
Guinea pig	Dorsal motor nucleus of vagus	123.0 (S.E. \pm 2.5, n = 6)	14.6%
Marmoset	Dorsal motor nucleus of vagus	No labelled cells found	

enough to enable quantification of the postganglionic distribution.

Parasympathetic innervation

From the 3 species studied, only the guinea pig demonstrated a clear parasympathetic innervation to the adrenal medulla (Table 2, Fig. 5c). This projection was in the same order of magnitude as that of the postganglionic sympathetic supply. In the rat and guinea pig the fibres arose from both the right and left

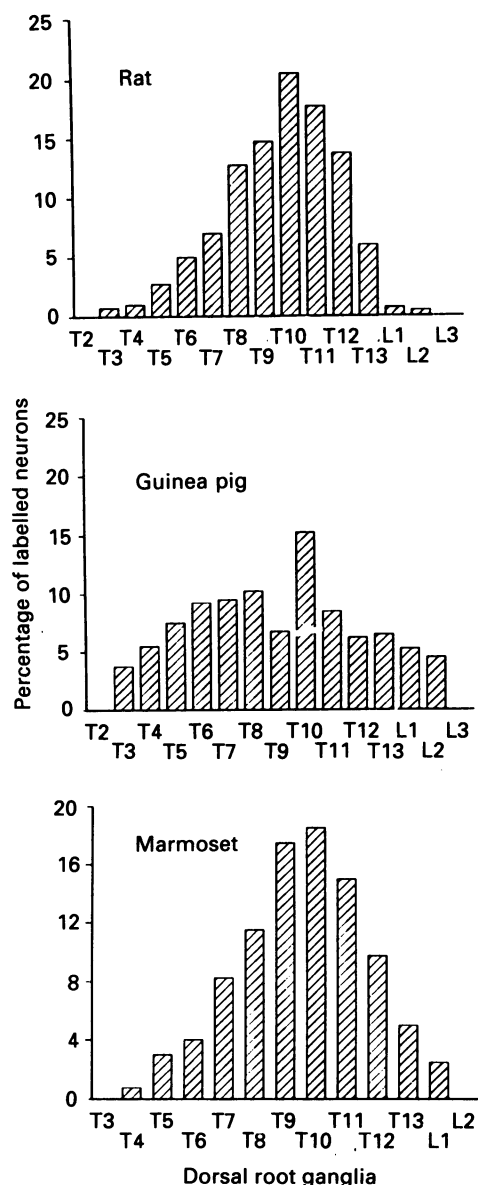


Fig. 7. Percentage contribution of fast blue-labelled neurons in the ipsilateral dorsal root ganglion after fast blue injections into the left adrenal gland of rat, guinea pig and marmoset.

Table 3. *Afferent innervation**

Rat		
Dorsal root ganglia	164.0 (S.E. \pm 10.6, n = 10)	86%
Vagal sensory ganglia	27.4 (S.E. \pm 2.2, n = 8)	14%
Guinea pig		
Dorsal root ganglia	503.0 (S.E. \pm 16.8, n = 8)	91%
Vagal sensory ganglia	49.0 (S.E. \pm 1.47, n = 8)	9%
Marmoset		
Dorsal root ganglia	85.0 (S.E. \pm 8.2, n = 5)	79%
Vagal sensory ganglia	18.3 (S.E. \pm 0.9, n = 5)	21%

dorsal motor nucleus of the vagus, although the labelling in the rat was very inconsistent. We were unable to find labelled cells in this nucleus in the

marmoset. Additionally, there was no evidence for a pelvic parasympathetic supply to the adrenal medulla in any of the 3 species studied.

Afferent innervation

The adrenal medulla of all 3 species studied received a significant afferent nerve supply. The cell bodies lay within the dorsal root ganglia (DRG) and the vagal sensory (nodose) ganglia (VSG). The labelling in the DRG was ipsilateral to the site of injection but was bilateral in the VSG. The distribution of labelled cell bodies in the DRG followed closely that of the efferent innervation (Fig. 7) with major contributions arising from ganglia at T9 and T10, although the distribution pattern was significantly broader in the guinea pig. The cells were between 6 to 36 μ m in diameter (Fig. 5*d*), the majority averaging 24 μ m in both DRG and VSG. Table 3 shows that in all 3 species the dominant source of afferent fibres to the adrenal medulla arose from the DRG, representing 91% in the guinea pig with respect to the total number of afferent cell bodies labelled. The total number of afferent fibres projecting to the marmoset adrenal medulla was considerably lower in comparison with the other 2 species.

DISCUSSION

The results of the fluorescent tracer studies outlined above provide unequivocal support for the early anatomical and physiological studies of the innervation of the adrenal gland. We can now see that the adrenal, like many other visceral organs, receives an extensive efferent nerve supply originating in both the sympathetic and parasympathetic nervous systems as well as in the afferent system and whose fibres run in both divisions of the peripheral autonomic nervous system.

It also demonstrates that essentially a similar pattern of innervation exists in both mammals, including primates. The source of any variation may be due simply to species differences but may also have a deeper foundation in the structure and function of the adrenals in the different animals. While it is true to say that the mammalian adrenal gland follows a common plan of a central core of medulla surrounded by cortex, ultrastructural examination of the medulla reveals differences in the proportion of the 2 main types of chromaffin cell. This difference is further manifested in the storage levels of the 2 main catecholamines. Since a large proportion of the fibres projecting to the adrenal gland is destined to innervate

chromaffin cells, the differing ratios of these cells in the 3 species may be considered to reflect the pattern of their innervation and thus form an anatomical substrate for the differential response of the adrenal medulla to noxious and nonnoxious stimuli (Ito et al. 1984). However, there appears no obvious link between the 2 apart from the fact that the relatively high level of parasympathetic innervation in the guinea pig (Parker et al. 1990) may be indicative of the considerably more adrenomedullary neurons found in this species as compared with the rat (Coupland, 1965). It may therefore reflect a preganglionic parasympathetic innervation of these cells. Also it is possible that there are distinct neural connections with cells bearing the different subtypes of cholinergic or peptidergic receptor. Alternatively, the differential release of catecholamines may occur either as a result of activation of different regions of the brainstem or hypothalamus (Folkow & von Euler, 1954) or, as has been shown by Edwards (1982), by altering the frequency of nerve impulses in the splanchnic nerve. Quite how this differential response is elicited peripherally is unclear. Whilst the lack of labelled cells in the nucleus of the vagus in the marmoset suggests a sparse neuronal population in the adrenal medulla, confirmation must await the results of a more detailed study which is underway in this Department. However, in general terms, the reduced labelling in the marmoset as compared with the rat and guinea pig may simply reflect the less efficient transportation of fluorescent neuronal tracers in this species.

Discovering the precise destination of fibres projecting to the adrenal gland is further complicated because of diffusion of tracer away from the injection site within the gland. Examination of the injection site revealed that although the focus of the injection was the centre of the medulla, and therefore terminals in this region would have the greatest exposure to tracer, the possibility cannot be excluded that low concentrations of tracer reached the corticomedullary junction by diffusion and also along the micropipette track. However, extensive labelling of the cortex is thought not to occur and therefore the results of our present studies should not be seen as definitive evidence for innervation of the adrenal cortex (Carlson et al. 1990). We would say that the majority of fibres of both efferent and afferent systems terminate within the medulla and that it is possible that a small proportion of these fibres may occur in regions of the cortex closest to the medulla. A further possibility is that cortex and medulla are supplied by the same fibres which, on piercing the capsule, branch extensively in subcapsular, cortical and medullary

regions. If this is true, then the pattern for cortical and medullary innervation would be similar. Indeed, it would appear from our studies that preganglionic AChE positive fibres branch appreciably, at least in the guinea pig. Also, the ratio of the total number of retrogradely labelled cells per animal to the number of synapses present on chromaffin cells clearly indicates extensive branching of the fibres (Tomlinson & Coupland, 1990). There may be species variation since both the rat and marmoset fibres destined for the medulla did not appear to branch extensively in the cortex. This is supported by the more detailed studies of Watanabe et al. (1990) on the goat, where it appears that AChE-positive nerve bundles run through the cortex to the medulla without branching apart from some ramification in the corticomedullary boundary. Clarification of these points must await further studies.

Significant clues as to the function of the different categories of nerve fibres projecting to the adrenal medulla have been provided by both functional and immunohistochemical studies. Extensive neuropeptide networks have been demonstrated in both the cortex and medulla of mammals including man (Holzwarth, 1984; Peltto-Huikko et al. 1985; Kondo et al. 1986; Bacon & Smith, 1988; Maubert, 1990; C. H. Heym & M. Colombo-Benkmann, unpublished observations). Livett and his group have accumulated convincing evidence to support the role of substance P-containing fibres in the modulation of catecholamine release from the adrenal medulla (Khalil et al. 1986; Livett, 1987; Livett et al. 1990). In addition, Mohamed et al. (1988) ascribed a possible sensory function to afferent inputs to the guinea pig adrenal on the basis of the presence of nerve terminals which resemble baroreceptors found in the carotid sinus (Bock & Gorgas, 1976; Gorgas et al. 1983). In support of this possibility it has been shown that the adrenal nerve is sensitive to baroreceptor activity in the adrenal medulla (Nijijima & Winter, 1968). Since most of these sensory-like endings are found either in the capsular region or associated with blood capillaries in both cortex and medulla, they may be part of a feedback circuit involved in monitoring capsular or vascular distension as a consequence of changes in blood pressure.

The role of the postganglionic fibres projecting to the adrenal medulla is more controversial since there has been no confirmation of the early reports by Prentice & Wood (1975) of direct adrenergic innervation of chromaffin cells in the cat adrenal medulla, nor have there been reports of adrenergic terminals innervating medullary vascular elements.

The level of postganglionic innervation is relatively low in all 3 species studied and perhaps therefore one should be cautious when ascribing a direct role of these fibres in catecholamine secretion, at least until a thorough reevaluation of the morphological evidence for adrenergic terminals in the medullas of these animals has been undertaken. However, the level of innervation is sufficient for it to be considered as playing a role in vascular control. As mentioned earlier, it is possible that tracer diffusion towards the corticomedullary junction could contaminate terminals present on vascular or cortical elements in this region. Indeed, physiological evidence is accumulating to support the postganglionic innervation of the adrenal cortex (Carlson et al. 1990) and in particular the vascular elements (Carlson et al. 1992). However, it should be reaffirmed that there is no evidence to suppose that these terminals are not simply a part of an extensive network of branches belonging to the same nerve fibres destined to innervate medullary vascular or chromaffin elements. In this case the pattern of labelling would be the same irrespective of where the terminals are located.

In conclusion, the fluorescent tracer studies confirm that the adrenal medulla, and possibly the corticomedullary junction, receive a multicomponent innervation. Clarification of the role of the different types of fibres is required and of the possible way in which the innervation of the cortex and medulla may be linked. This will lead to a better understanding of the mechanism of adrenal corticomedullary responses to internal and external stimuli and their control by both local circuits and higher centres in the CNS.

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REFERENCES

- AFEWOK M (1988) *The Sensory Innervation of the Rat Adrenal Gland*. M. Phil. Thesis, University of Nottingham.
- ALPERT LK (1931) Innervation of suprarenal gland. *Anatomical Record* **50**, 221–234.
- BACON SJ, SMITH AD (1988) Preganglionic sympathetic neurones innervating the rat adrenal medulla: immunocytochemical evidence of synaptic input for nerve terminals containing substance P, GABA or 5-hydroxytryptamine. *Journal of the Autonomic Nervous System* **24**, 97–122.
- BOCK P, GORGAS K (1976) The fine structure of baroreceptor terminals in the carotid sinus of guinea pigs and mice. *Cell and Tissue Research* **170**, 95–112.
- BORNSTEIN SR, EHRHART-BORNSTEIN M, SCHERTBAUM WA, PFEIFFER EF, HOLST JJ (1990) Effects of splanchnic nerve stimulation on the adrenal cortex may be mediated by chromaffin cells in a paracrine manner. *Endocrinology* **127**, 900–906.
- CARLSON S, SKARPHEDINSSON JO, JENNISCHE E, DELLE M, THOREN P (1990) Neurophysiological evidence for and characterization of the postganglionic innervation of the adrenal gland in the rat. *Acta Physiologica Scandinavica* **140**, 491–499.
- CARLSON S, SKARPHEDINSSON JO, DELLE M, HOFFMAN P, THOREN P (1992) Reflex changes in post and preganglionic sympathetic adrenal nerve activity and postganglionic renal nerve activity upon arterial baroreceptor activation and during severe haemorrhage in the rat. *Acta Physiologica Scandinavica* **144**, 317–323.
- CELLER B, SCHRAMM LP (1981) Pre- and postganglionic sympathetic nerves of the rat. *American Journal of Physiology* **241**, R55–R61.
- CHARLTON BC (1990) Adrenal cortical innervation and glucocorticoid secretion. *Journal of Endocrinology* **126**, 5–8.
- COUPLAND RE (1965) *The Natural History of the Chromaffin Cell*. London: Longmans.
- COUPLAND RE (1975) Blood supply of the adrenal gland. In *Handbook of Physiology*, Section 6, *Endocrinology* (ed. H. Blaschko, G. Sayers & A. D. Smith), pp. 283–294. Washington, D.C.: American Physiological Society.
- COUPLAND RE, HOLMES RL (1958) The distribution of cholinesterase in the adrenal glands of the rat, cat and rabbit. *Journal of Physiology* **141**, 97–106.
- COUPLAND RE, SELBY JE (1976) The blood supply of the mammalian adrenal medulla: a comparative study. *Journal of Anatomy* **122**, 539–551.
- COUPLAND RE, PARKER TL, KESSE WK, MOHAMED AA (1989) The innervation of the adrenal gland. III. Vagal innervation. *Journal of Anatomy* **163**, 173–181.
- DOGIEL AS (1894) Die Nervenendigungen in den Nebennieren der Säugetiere. *Archiv für Anatomie und Physiologie, Leipzig*, 90–104.
- DOUGLAS WW, POISNER AM (1965) Preferential release of adrenaline from the adrenal medulla by muscarine and pilocarpine. *Nature* **208**, 1102–1103.
- EDWARDS AV (1982) Adrenal catecholamine output in response to stimulation of the splanchnic nerve in bursts in the conscious calf. *Journal of Physiology* **327**, 409–419.
- EDWARDS AV (1990) Autonomic control of endocrine pancreas and adrenal function. In *Central Regulation of Autonomic Functions* (ed. A. D. Loewy & K. M. Spyer), pp. 286–309. Oxford: Oxford University Press.
- ELLIOT TR (1913) The innervation of the adrenal glands. *Journal of Physiology* **46**, 285–290.
- ELLISON JP, CLARK GM (1975) Retrograde axonal transport of horseradish peroxidase in peripheral autonomic nerves. *Journal of Comparative Neurology* **161**, 103–114.
- FELDBERG W, MINZ B, TSUDZIMURA H (1934) Mechanism of nervous discharge of adrenaline. *Journal of Physiology* **81**, 286–304.
- FOLKOW B, VON EULER US (1954) Selective activation of noradrenaline and adrenaline producing cells in the cat's adrenal gland by hypothalamic stimulation. *Circulation Research* **2**, 191–195.
- GARCIA-ALVEREZ F (1972) Características ultraestructurales y significación de las vesículas sinápticas adrenérgicas de la corteza suprarenal delcavia cobaya. *Anales de Anatomía (Zaragoza)* **21**, 301–310.
- GOADSBY PJ (1985) Brainstem activation of the adrenal medulla in the cat. *Brain Research* **327**, 241–248.
- GORGAS K, REINECKE M, WEIH E, FORSMANN WG (1983) Neurotensin and substance-P immunoreactive nerve endings in

- the guinea pig carotid sinus and their ultrastructural counterparts. *Anatomy and Embryology* **167**, 347–354.
- GORNE RC, PFISTER C, RATHSACK R, OEHME P (1984) Substance P-like immunoreactivity in rat adrenal medulla. *Biomedical et Biochimica Acta* **43**, 135–137.
- HAASE P, CONTESTABILE A, FLUMERFELT BA (1982) Preganglionic innervation of the adrenal gland of the rat using horse-radish peroxidase. *Experimental Neurology* **78**, 217–221.
- HEXUM TD, BARRON BA (1984) Met5-enkephalin-like peptide release from the adrenal gland. In *Dynamics of Neurotransmitter Function* (ed. I. Hanin), pp. 137–142. New York: Raven Press.
- HINSON JP (1990) Paracrine control of adrenocortical function: a new role for the medulla? *Journal of Endocrinology* **124**, 7–9.
- HÖKFELT T, LUNDBERG JM, SCHULTZBERG M, FAHRENKRUG J (1981) Immunohistochemical evidence for a local VIP-ergic neuron system in the adrenal gland of the rat. *Acta Physiologica Scandinavica* **113**, 575–576.
- HOETS V, ELDE R (1982) The differential distribution and relationship of serotonergic and peptidergic fibres to the sympathoadrenal neurons in the intermediolateral cell column of the rat: a combined retrograde axonal transport and immunofluorescence study. *Neuroscience* **7**, 1155–1174.
- HOLLINSHEAD WH (1936) The innervation of the adrenal glands. *Journal of Comparative Neurology* **64**, 449–467.
- HOLLINSHEAD WH (1937) The innervation of abdominal chromaffin tissue. *Journal of Comparative Neurology* **67**, 133–143.
- HOLZWARTH MA (1984) The distribution of vasoactive intestinal peptide in the rat adrenal cortex and medulla. *Journal of the Autonomic Nervous System* **11**, 269–283.
- HOLZWARTH MA, CUNNINGHAM LA, KLEITMAN N (1987) The role of the adrenal nerves in the regulation of adrenal cortical functions. *Annals of the New York Academy of Sciences* **512**, 449–464.
- HOSHI T (1926) Morphologisch-experimentelle Untersuchung über die Innervation der Nebenniere. *Mitteilung für allgemeine Pathologie Sendai* **3**, 328–342.
- ITO K, SATO A, SHIMAMURA K, SWENSON RS (1984) Reflex changes in sympatho-adrenal medullary functions in response to baroreceptor stimulation in anaesthetized rats. *Journal of the Autonomic Nervous System* **10**, 259–303.
- JONES CT, EDWARDS AV (1991) Muscarinic adrenal responses to acetylcholine in conscious calves. *Journal of Physiology* **444**, 605–614.
- KARNOVSKY MJ, ROOTS L (1964) A direct-colouring thiocholine method for cholinesterase. *Journal of Histochemistry and Cytochemistry* **12**, 219–221.
- KATAFUCHI T, YOSHIMATSU H, OOMURA Y, SATO A (1986) Responses of adrenal catecholamine secretion to lateral hypothalamic stimulation and lesion in rats. *Brain Research* **363**, 141–144.
- KESSE WF (1988) *Innervation of the Rat Adrenal Gland*. PhD Thesis, University of Nottingham.
- KESSE WF, PARKER TL, COUPLAND RE (1988) The innervation of the adrenal gland. I. The source of pre- and postganglionic nerve fibres to the rat adrenal gland. *Journal of Anatomy* **157**, 33–41.
- KHALIL Z, LIVETT BG, MARLEY PD (1986) The role of sensory fibres in the rat splanchnic nerve in the regulation of adrenal medullary secretion during stress. *Journal of Physiology* **370**, 201–215.
- KIKUTA A, MURAKAMI T (1982) Microcirculation of the rat adrenal gland: a scanning electron microscope study of vascular casts. *American Journal of Anatomy* **164**, 19–28.
- KIKUTA A, MURAKAMI T (1984) Relationship between chromaffin cells and blood vessels in the rat adrenal medulla: a transmission electron microscope study combined with blood vessel reconstructions. *American Journal of Anatomy* **170**, 73–81.
- KISS T (1951) Experimental-morphologische Analyse der Nebenniereninnervation. *Acta Anatomica* **13**, 81–89.
- KLEITMAN N, HOLZWARTH MA (1985) Catecholaminergic innervation of the rat adrenal cortex. *Cell and Tissue Research* **241**, 139–247.
- KNIGHT DE, BAKER PF (1986) Observations on the muscarinic activation of catecholamine secretion in the chick adrenal. *Neuroscience* **19**, 357–366.
- KOBAYASHI S, OHASHI T, UCHIDA T, YANAIHARA N (1983) Met-enkephalin-arg-gly-leu-like immunoreactivity in presynaptic nerve terminals on both adrenaline-storing (A) and noradrenaline-storing (NA) cells of the rat adrenal medulla. *Biomedical Research* **4**, suppl., 151–158.
- KOBAYASHI S, MIYABAYASHI T, UCHIDA T, YANAIHARA N (1985) Met-enkephalin-arg-gly7-leu8 in large cored vesicles of splanchnic nerve terminals innervating guinea pig adrenal chromaffin cells. *Neuroscience Letters* **53**, 247–252.
- KONDO H, KURAMOTO H, FUJITA T (1986) An immuno-electron-microscope study of the localization of VIP-like immunoreactivity in the adrenal gland of the rat. *Cell and Tissue Research* **245**, 531–538.
- KUYPERS HGJM, HUISMAN AM (1984) Fluorescent neuronal tracers. In *Advances in Cellular Neurobiology*, vol. 5, *Labeling Methods Applicable to the Study of Neuronal Pathways*, pp. 307–340. New York: Academic Press.
- LE DOUARIN NM (1982) *The Neural Crest*. Cambridge: Cambridge University Press.
- LEWIS PR, SHUTE CCD (1969) An electronmicroscopic study of cholinesterase distribution in the rat adrenal medulla. *Journal of Microscopy* **89**, 181–193.
- LIANG BT, PERLMAN RL (1979) Catecholamine secretion by hamster adrenal cells. *Journal of Neurochemistry* **32**, 927–933.
- LIVETT BG (1987) Peptide modulation of adrenal chromaffin cell secretion. In *Stimulus Secretion Coupling in Chromaffin Cells* (ed. K. Rosenheck & P. I. Lelkes), pp. 117–150. Boca Raton, Florida: CRC Press.
- LIVETT BG, MARLEY PD, WAN DC-C, ZHOU X-F (1990) Peptide regulation of adrenal medullary function. *Journal of Neural Transmission* **29**, suppl, 77–89.
- MALMEJAC (1964) Activity of the adrenal medulla and its regulation. *Physiological Reviews* **44**, 186–218.
- MARLEY PD, LIVETT BG (1985) Neuropeptides in the autonomic nervous system. *CRC Critical Reviews in Clinical Neurobiology* **1**, 201–283.
- MARLEY PD, BUNN SJ, WAN DC-C, ALLEN AM, MENDELSON FAO (1989) Localization of angiotensin II binding sites in the bovine adrenal medulla using a labelled specific antagonist. *Neuroscience* **28**, 777–787.
- MATSUI H (1984) Adrenal medullary secretion in response to diencephalic stimulation in the rat. *Neuroendocrinology* **38**, 164–168.
- MAUBERT E, TRAMU G, CROIX D, BEAUVILLAIN JC, DUPOUY JP (1990) Co-localization of vasoactive intestinal polypeptide and neuropeptide Y immunoreactivities in the nerve fibres of the rat adrenal gland. *Neuroscience Letters* **113**, 121–126.
- MIGALLY N (1979) Innervation of the mouse adrenal cortex. *Anatomical Record* **194**, 105–112.
- MOHAMED A, PARKER TL, COUPLAND RE (1988) The innervation of the adrenal gland. II. The source of spinal afferent nerve fibres to the guinea pig adrenal gland. *Journal of Anatomy* **160**, 51–58.
- NIJIMA A, WINTER AL (1968) The effect of catecholamines on unit activity in afferent nerves from the adrenal glands. *Journal of Physiology* **195**, 647–656.
- OOMORI Y, OKUNO S, FUJASAWA H, ONO K (1991) Immunoelectron microscopic study of tyrosine hydroxylase immunoreactive nerve fibres and ganglion cells in the rat adrenal gland. *Anatomical Record* **229**, 407–414.
- PARKER TL, AFEWORK M, COUPLAND RE (1990a) Sensory innervation of the rat adrenal gland. *Neuroscience Letters* **38**, 562.
- PARKER TL, MOHAMED AA, COUPLAND RE (1990b) The inner-

- vation of the adrenal gland. IV. The source of pre- and post-ganglionic nerve fibres to the guinea pig adrenal gland. *Journal of Anatomy* **172**, 17–24.
- PELTO-HUIKKO M, SALMINEN T, HERVONEN A (1985) Localization of enkephalins in adrenaline cells and the nerves innervating adrenaline cells in rat adrenal medulla. *Histochemistry* **82**, 377–383.
- PFISTER VC, GORNE RC (1983) Substance-P-like immunofluorescence in the adrenal medulla of the rat. *Acta Histochemica (Jena)* **72**, 127–129.
- PIEZZI R (1966) Two types of synapses in the chromaffin tissue of the toad's adrenal. *Acta Physiologica Latinoamericana* **16**, 282–285.
- PINES L, NAROWTSCHATOWA K (1931) Über die Innervation des chromaffin Gewebes des Sympathicus und über das sympathico-chromaffin System in allgemeinen. *Archiv für Psychiatrie und Nervenkrankheiten* **70**, 636–647.
- PRENTICE FD, WOOD JG (1975) Adrenergic innervation of the cat adrenal medulla. *Anatomical Record* **181**, 689–704.
- ROBINSON PM, PERRY RA, HARDY KJ, COGLAN JP, SCOGGINS BA (1977) The innervation of the adrenal cortex in the sheep *Ovis ovis*. *Journal of Anatomy* **124**, 117–129.
- ROBINSON RL, CULBERSON JL, CARMICHAEL SW (1983) Influence of hypothalamic stimulation on the secretion of adrenal medullary catecholamines. *Journal of the Autonomic Nervous System* **8**, 89–96.
- ROLE LW, PERLMAN RL (1983) Both nicotinic and muscarinic receptors mediate catecholamine secretion by isolated guinea-pig chromaffin cells. *Neuroscience* **10**, 979–985.
- RUBIN RP, MIELE A (1968) A study of the differential secretion of epinephrine and norepinephrine from the perfused cat adrenal gland. *Journal of Pharmacology and Experimental Therapeutics* **164**, 115–121.
- SCHNEIDER A (1987) Muscarinic receptor mechanism in adrenal chromaffin cells. In *Stimulus-Secretion Coupling in Chromaffin Cells* (ed. K. Rosenheck & P. I. Lelkes), pp. 51–86. Boca Raton, Florida: CRC Press.
- SCHRAMM LP, ADAIR JR, STRIBLING JM, GREY LP (1975) Preganglionic innervation of the adrenal gland of the rat: a study using horseradish peroxidase. *Experimental Neurology* **49**, 540–553.
- SCHULTZBERG M, LUNDBERG JM, HÖKFELT T, TERENIUS L, BRANDT J, ELDE RP, GOLDSTEIN M (1978) Enkephalin-like immunoreactivity in gland cells and nerve terminals of the adrenal medulla. *Neuroscience* **3**, 1169–1186.
- SHIODA T, HISHIDA S (1967) The innervation of the adrenal cortex. *Archivum Histologicum Japonicum* **28**, 23–43.
- SPARROW RA, COUPLAND RE (1987) Blood flow to the adrenal gland of the rat: its distribution between the cortex and the medulla before and after haemorrhage. *Journal of Anatomy* **155**, 51–61.
- STODDARD-APTER SL, SIEGEL A, LEVIN BE (1983) Plasma catecholamine and cardiovascular responses following hypothalamic stimulation in the awake cat. *Journal of the Autonomic Nervous System* **8**, 343–360.
- STRACK AM, SAWYER WB, MARUBIO LM, LOEWY AD (1988) Spinal origin of sympathetic preganglionic neurons in the rat. *Brain Research* **455**, 187–191.
- STRACK AM, SAWYER WB, PLATT KB, LOEWY AD (1989) CNS cell groups regulating the sympathetic outflow to adrenal gland as revealed by transneuronal cell body labelling with pseudorabies virus. *Brain Research* **491**, 274–296.
- SWINYARD CA (1937) The innervation of the suprarenal glands. *Anatomical Record* **68**, 417–428.
- TETLEBAUM HA (1933) The nature of the thoracic and abdominal distribution of the vagus nerves. *Anatomical Record* **55**, 297–317.
- TOMLINSON A, COUPLAND RE (1990) The innervation of the adrenal gland. IV. Innervation of the rat adrenal medulla from birth to old age. A descriptive and quantitative morphometric and biochemical study of the innervation of chromaffin cells and adrenal medullary neurons in Wistar rats. *Journal of Anatomy* **169**, 209–236.
- UNSICKER K (1971) On the innervation of the rat and pig adrenal cortex. *Zeitschrift für Zellforschung* **116**, 151–156.
- UNSICKER K (1984) Innervation of endocrine tissues. In *Ultrastructure of Endocrine Cells and Tissues* (ed. P. M. Motta), pp. 321–332. The Hague: Martinus Nijhoff.
- UNSICKER K, HABURA-FLUH O, ZWARG U (1978) Different types of small granule-containing cells and neurons in the guinea-pig adrenal medulla. *Cell and Tissue Research* **189**, 109–130.
- VARNDELL IM, POLAK JM, ALLEN JM, TERENGI G, BLOOM SR (1984) Neuropeptide tyrosine (NYP) immunoreactivity in norepinephrine-containing cells and nerves of the mammalian adrenal gland. *Endocrinology* **114**, 1460–1462.
- VERHOFSTADT AAJ, COUPLAND RE, PARKER TL, GOLDSTEIN M (1985) Immunohistochemical and biochemical study on the development of the noradrenaline- and adrenaline-storing cells of the adrenal medulla of the rat. *Cell and Tissue Research* **242**, 233–243.
- WAKADE AR, WAKADE TD (1983) Contribution of nicotinic and muscarinic receptors in the secretion of catecholamines evoked by endogenous and exogenous acetylcholine. *Neuroscience* **10**, 973–978.
- WATANABE T, HIRAMATSU K, OHMORI Y, PAIK Y-K (1990) Histo- and cytochemical studies on the distribution of acetylcholinesterase-positive nerve fibres in the goat adrenal gland. *Anatomia, Histologia, Embryologia* **19**, 245–254.
- WESSELINGH S, LI Y, BLESSING WW (1989) PNMT-containing neurones in the rostral medulla oblongata C1,C3 groups are transneuronally labelled after injection of herpes simplex virus type 1 into the adrenal gland. *Neuroscience Letters* **106**, 99–104.
- WILLARD DM (1936) The innervation of the adrenal glands of mammals; a contribution to the study of nerve-endings. *Quarterly Journal of Microscopical Science* **78**, 475–485.
- YOUNG JZ (1939) Partial degeneration of the nerve supply of the adrenal. A study in autonomic innervation. *Journal of Anatomy* **73**, 540–550.
- ZHOU S-F, LIVETT BG (1990) Substance P increases catecholamine secretion from perfused rat adrenal glands evoked by prolonged field stimulation. *Journal of Physiology* **425**, 321–334.
- ZHOU S-F, OLDFIELD BJ, LIVETT BG (1991) Substance-P containing sensory neurons in the rat dorsal root ganglia innervate the adrenal medulla. *Journal of the Autonomic Nervous System* **33**, 247–254.