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The innervation of the adrenal gland. IV. The source of pre- and postganglionic nerve fibres to the guinea-pig adrenal gland

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INTRODUCTION

The accepted view of the efferent innervation to the adrenal gland is that of preganglionic sympathetic neurons located in the thoracolumbar segments of the spinal cord which project to the adrenal gland via the splanchnic nerve (Elliot, 1913; MacFarland & Davenport, 1941; Coupland, 1965). Although the reports of Young (1933) and Swinyard (1937) provided strong evidence for an additional postganglionic innervation to the adrenal gland, recent reports, using neuronal fibre tracing techniques, have not attempted to prove or disprove these earlier claims (Schramm, Adair, Stribling & Gray, 1975; Holets & Elde, 1982). However more recently, Kesse, Parker & Coupland (1988) have provided conclusive evidence, using the retrograde tracer Fast Blue, that the rat adrenal gland does receive both a pre- and a postganglionic innervation. They found that 11·4% of neurons projecting to the adrenal gland were postganglionic arising in the sympathetic chain and suprarenal ganglion; the remainder were preganglionic, arising from the intermediolateral horn of the spinal cord segments T1–L1.

Ellison & Clark (1975) reported that labelled cells were found in the spinal cord after injections of horseradish peroxidase into the guinea-pig adrenal medulla. However, the distribution pattern of labelled neurons was not reported. In the present work we have searched for both the source of preganglionic nerve fibres to the guinea-pig adrenal gland and for any postganglionic innervation similar to that found in the rat.

MATERIALS AND METHODS

A total of fifteen adult guinea-pigs (380–420 g) of both sexes were used in this study. The animals were kept under standard laboratory conditions with food and water *ad libitum*.

The animals were anaesthetised by a combination of Hypnorm (Phentanyl citrate, 0·315 mg and fluaisone, 10 mg per ml) and Diazepam (Valium, 5 mg per ml). The Diazepam (2·5 mg/kg) and Hypnorm (1 ml/kg) were both administered by intramuscular injection according to Green (1982).

The procedure for the injection of tracers (Fast Blue or WGA-HRP) into the adrenal gland followed closely that of Mohamed, Parker & Coupland (1988). Briefly, a midline incision was made through the skin and ventral abdominal muscles. After opening the peritoneum, the intestine, stomach and spleen were gently retracted allowing free access to the left adrenal gland. A 2% aqueous suspension of Fast Blue (Dr Illing Makromolekulare Chemie, Gross-Umstadt, FRG) or a 20% solution of

WGA-HRP in saline was slowly pressure injected (5 μl) into the adrenal medulla over a period of 15 minutes via a glass micropipette (tip diameter 20 μl) coupled to a 25 μl Hamilton syringe.

After tracer delivery the micropipette remained in the gland for 5 minutes and was subsequently withdrawn over a further period of 15 minutes to allow tracer to drain from the tip and to be taken up by the medullary tissue. The entry point of the micropipette into the capsule was sealed by electrical cauterisation.

Following the suture of the anterior body wall muscles, an intramuscular injection of Penbritin (100 μl of a 1 ml suspension containing 150 mg ampicillin trihydrate) was administered. The animals were allowed to recover and then returned to the animal house.

After seven days (Fast Blue injections) or four days (WGA-HRP injections), the guinea-pigs were anaesthetised with an intraperitoneal injection of sodium pentobarbitone (60 mg/kg) and fixed by transcardiac perfusion in the following sequence: prewash containing 3% dextran 70 and 1% procaine hydrochloride at body temperature for 1 minute at a pressure of 120 mmHg, followed by 500 ml of either 4% freshly prepared paraformaldehyde in 0·1 M phosphate buffer pH 7·4, for Fast Blue injected animals, or a mixture of 1·25% glutaraldehyde and 1% paraformaldehyde in 0·1 M phosphate buffer pH 7·4 for WGA-HRP injected animals. Both were perfused at a pressure of 10 mmHg at 4 °C, and followed by 200 ml of 10% sucrose buffer at 4 °C also at a pressure of 120 mmHg.

The thoracic and upper lumbar segments of the spinal cord as well as the paravertebral and prevertebral ganglia were dissected out as described by Kesse *et al.* (1988). The tissues were immersed in the same fixative for 90 minutes and rinsed in 0·1 M phosphate buffer (pH 7·4) containing 10% sucrose for 24 hours at 4 °C. Serial cryostat sections (20 μm) of the spinal cord were cut, either in the longitudinal (dorsoventral) or transverse plane. The pre- and paravertebral ganglia were embedded in plastic (JB-4) and serial sections were cut at 10 μm.

The sections prepared from WGA-HRP injected animals were processed according to Mesulam (1978) using the tetramethylbenzidine technique; sections prepared from Fast Blue injected animals were examined using a Leitz Ortholux II microscope fitted with a Ploempack fluorescent system and an excitation wavelength of 360 nm. They were photographed using either Ilford Pan-f 135 or Kodak Ektachrome (200 ASA) film respectively. The location, shape and size of the labelled neurons were noted. To avoid double counting, only those cells where the nucleus was visible were recorded.

Tracer injection into a highly vascular organ such as the adrenal gland raises the possibility of leakage into the vascular system resulting in false positive labelling. Injections of 5 μl of either Fast Blue or WGA-HRP into the guinea-pig circulation via the tail vein resulted in a total absence of labelling in all the tissues (brain, spinal cord and peripheral autonomic nervous sysem) examined in this study.

RESULTS

Before searching for retrogradely labelled cells, the adrenal gland and surrounding tissues were carefully and routinely examined for tracer deposition. Data were collected only from those animals in which the tracer was confined to the adrenal gland. In those rare cases where fluorescence or a blue coloration was detected on the surface of the adrenal capsule or in the surrounding tissues, the animals were rejected. Examination of sections of the adrenal glands of Fast Blue and WGA-HRP injected animals, after 4 and 7 days, revealed that the tracer deposition was greatest in the

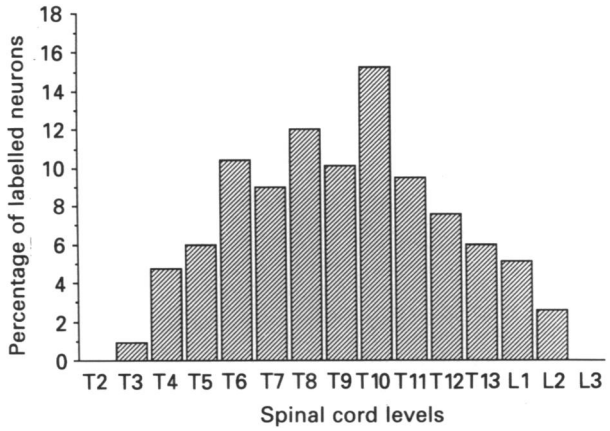


Fig. 1. Percentage contribution of Fast Blue labelled neurons in the spinal cord after injection of tracer into the left adrenal gland.

adrenal medulla. However, as a result of diffusion away from the injection site, regions of the inner cortex and those adjacent to the micropipette track as far as the inner part of the zona glomerulosa also contained the tracers.

Distribution of labelled cells

Preganglionic innervation. Spinal cord

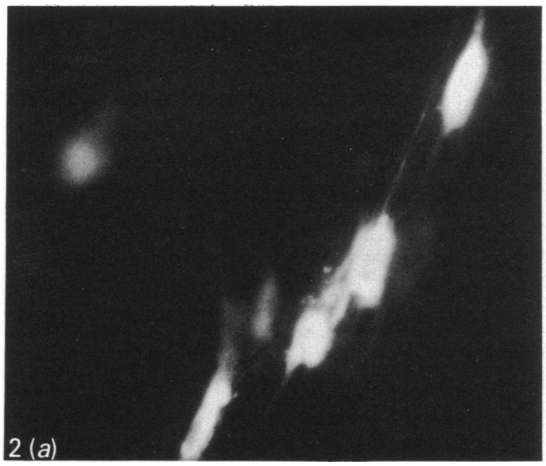
(*i*) *Fast Blue*. Retrogradely labelled neurons were found in the nucleus intermediolateralis pars principalis (ILP) of the spinal cord between segments T3–L2 ipsilateral to the site of injection into the left adrenal gland. They formed a column of cells at the lateral horn–lateral funicular border with cell clusters occurring within each spinal cord segment. The Fast Blue neurons appeared as icy-blue fluorescent cells with silver coloured cytoplasmic granules and unstained nuclei. They ranged in size and shape from fusiform (8×22 μm) to round (14 μm in diameter). Axons and dendrites could often be seen arising from the perikarya (Fig. 2). Occasionally, neurons were found in the white matter lateral to ILP in the nucleus intermediolateralis pars funicularis (ILF) and in the nucleus intercalatus (IC). The labelled cells were confined to segments T3–L2 and were not found on the contralateral side of the spinal cord.

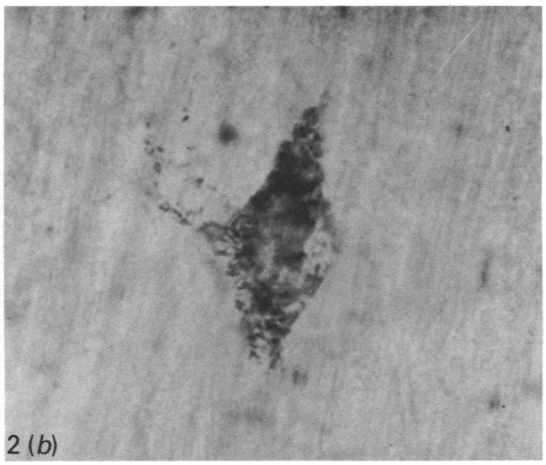
The distribution of FB retrogradely labelled neurons in the spinal cord is illustrated in Figure 1. This shows that 73·9% of the preganglionic neurons lie between segments T6–T12, and 46·4% of these neurons lie within segments T7–T10, the highest contribution coming from T10 (15·2%), representing $80 \pm 2{\cdot}3$ (SEM, n = 8).

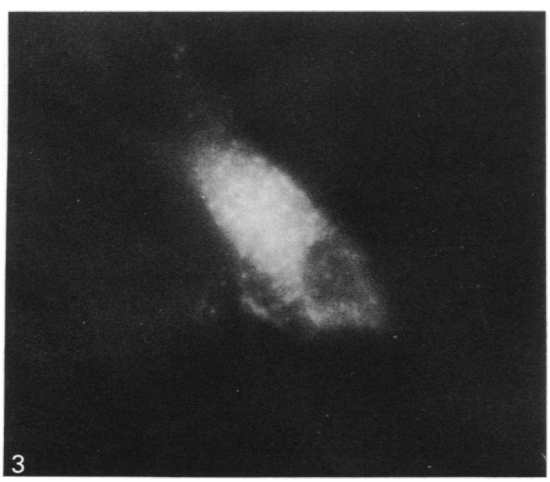
(*ii*) *WGA-HRP*. The preganglionic neurons projecting to the adrenal gland retrogradely labelled with WGA-HRP showed the same pattern of distribution in the spinal cord segments as the Fast Blue-containing preganglionic neurons. The WGA-HRP labelled neurons showed cytoplasmic reaction product but no tracer was found in the nuclei. The shape and size of these neurons was identical to those of the fluorescently labelled cells. The dense deposition of the reaction product in many neurons made visible their dendritic orientation. Many dendrites exhibited branching near their soma. Long dendrites were usually orientated in a mediolateral direction (Fig. 2).

Postganglionic innervation

(*i*) *Paravertebral ganglia*. Retrogradely labelled neurons were found in the thoracic ganglia of the paravertebral trunk ipsilateral to the site of injection of tracer into the







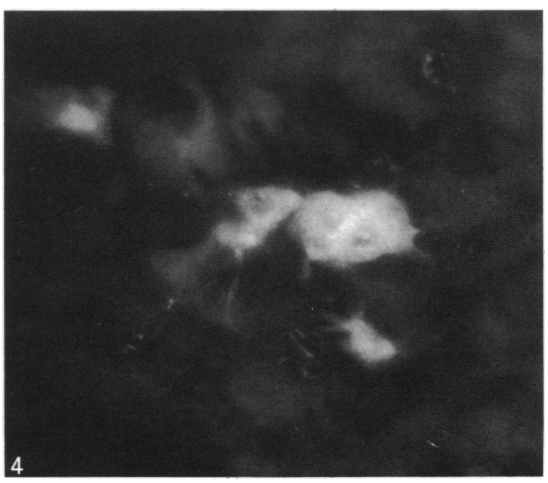


Fig. 2(*a–b*). Horizontal sections through the spinal cord showing (*a*) Fast Blue and (*b*) WGA-HRP labelled cells in the ILp nucleus. (*a*) ×350; (*b*) ×882.

Fig. 3. A Fast Blue labelled paravertebral ganglion cell in the left sympathetic chain. ×882.

Fig. 4. Fast Blue labelled neurons in the suprarenal ganglion. ×350.

left adrenal gland, from the first to the tenth thoracic sympathetic ganglia. The Fast Blue labelled neurons were oval in shape and their size ranged between 22–35 μm in the long and 14–20 μm in the short axis (Fig. 3). No labelled cells were found in the right paravertebral trunk. From Figure 5 it can be seen that the majority of postganglionic neurons projecting to the adrenal gland lay within the 9th–10th thoracic sympathetic ganglia, representing 60·2%. The greatest contribution arose from the 10th sympathetic ganglion (17·4%) representing $22 \pm 1{\cdot}0$ (SEM, n = 5) cells. A mean total of $125 \pm 3{\cdot}4$ (SEM, n = 5) retrogradely labelled postganglionic neurons were found per sympathetic chain.

(*ii*) *Prevertebral ganglia*

(*a*) *Suprarenal ganglion.* Typical FB labelled neurons were seen in the left suprarenal ganglion after injection of the left adrenal gland. The labelled neurons formed clusters of cells within the ganglion. The size and shape of these neurons ranged from oval

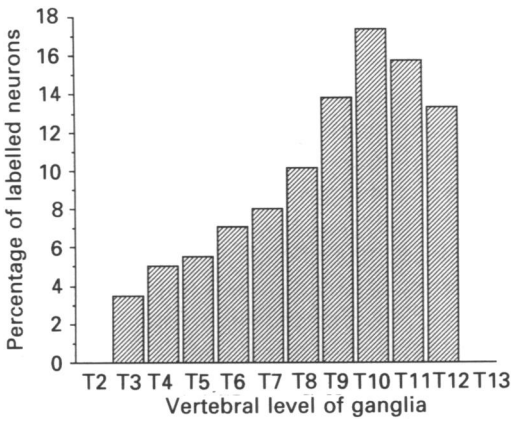


Fig. 5. Percentage contribution of Fast Blue labelled neurons in the ganglia of the left paravertebral sympathetic chain.

(30 × 18 μm) to round, 15 μm in diameter (Fig. 4). A mean total of 40 ± 1·5 (SEM) neurons was labelled in the 5 animals studied.

(*b*) *Coeliac ganglia.* Examination of the coeliac ganglia after injection of FB or WGA-HRP into the left adrenal gland revealed no labelled neurons present in these ganglia.

Examination of para- and prevertebral ganglia of WGA-HRP injected animals showed the labelled neurons to be morphologically similar and they had a similar distribution to that of the Fast Blue labelled neurons.

DISCUSSION

The results of this study provide conclusive evidence that the guinea-pig adrenal gland receives both a pre- and postganglionic sympathetic innervation. Although Ellison & Clark (1975) briefly referred to the preganglionic sympathetic innervation of the guinea-pig adrenal gland, the present report is the first to reveal the source and distribution of preganglionic nerve cell bodies projecting to the guinea-pig adrenal gland. In addition it also provides clear evidence for, and the source of, a postganglionic sympathetic innervation. The results are similar to those of Kesse *et al.* (1988) which demonstrated that the rat adrenal gland received both a pre- and postganglionic sympathetic innervation.

The entirely ipsilateral nature of the labelling in this study provides good evidence against the possibility that the neurons were labelled as a result of diffusion of tracer into the blood circulation, or of leakage and spread of tracer from the injection site into the surrounding tissues. This conclusion is strengthened by the fact that 5 μl of tracer injected into the circulatory system resulted in no labelling of cells in the brain, spinal cord or autonomic ganglia. Additionally, it was found that deliberately causing leakage of tracer into tissues surrounding the rat adrenal gland produced bilateral labelling of the spinal cord (Kesse *et al.* 1988).

In the present study, both Fast Blue and WGA-HRP retrogradely labelled neurons were consistently found ipsilateral to the site of injection. These results agree with previous reports in the cat (Marley & Prout, 1968), the dog (Cummings, 1969), the rat (Shramm *et al.* 1975; Kesse *et al.* 1988) and the guinea-pig (Ellison & Clark, 1975). It has been claimed by Ross, Smollen & Cherry (1981) that a contralateral preganglionic sympathetic innervation of the adrenal gland exists in the newborn rat. No evidence

to support these claims could be found by Parker, Kesse, Tomlinson & Coupland (1988) who concluded that innervation was ipsilateral from birth. Since the chance of tracer spread is greater in such small animals the contralateral labelling observed by Ross *et al.* (1981) may have been due to this fact.

The majority of labelled neurons were found within the intermediolateral column (ILp) in the nucleus intermediolateralis thoracolumbalis pars principalis (nomenclature according to Petras & Cummings, 1972). Occasionally retrogradely labelled neurons were found outside the ILp. These latter cells were observed in the lateral funiculus and probably belonged to the nucleus intermediolateralis thoracolumbalis pars funicularis, and the remainder to the nucleus intermediolateralis thoracolumbalis pars principalis and to the nucleus intercalatus spinalis. The distribution of labelled preganglionic neurons in the guinea-pig spinal cord was broadly similar to that found in the rat (Kesse *et al.* 1988), the greatest contributions arising from segments T7–T10 in the rat and T6–T12 in the guinea-pig. However, it differed in that in the rat the major contribution comes from T10 and in the guinea-pig from T9. Additionally, fewer labelled cells were found outside the ILp in the guinea-pig than in the rat.

It is clear that the guinea-pig adrenal gland receives a significant postganglionic innervation, representing 23% of the total projection to the adrenal gland. This is double the number of postganglionic neurons projecting to the rat adrenal gland (Kesse *et al.* 1988). The majority (75%) of postganglionic neurons in the guinea-pig arise from the sympathetic chain, the highest contribution coming from the ganglion at T10, while in the rat the greatest contribution is spread over sympathetic ganglia T9–T10. It is not known how these postganglionic fibres reach the adrenal gland but results of Kyo, Krauthamer & Yamasaki (1981) indicate that a substantial number of paravertebral postganglionic sympathetic neurons, with cell bodies situated mainly in ganglion T13, send fibres to join the major splanchnic nerve in the rat. These authors also found that the splanchnic postganglionic sympathetic fibres are virtually all unmyelinated axons in the major splanchnic nerve.

That this is not the only route for postganglionic fibres to reach the adrenal gland is supported by the fact that Kleitman & Holzwarth (1985) reported the persistence of catecholamine-containing nerve fibres in the rat adrenal gland after splanchnic nerve section. This finding is in agreement with the present results of labelled cells in the suprarenal ganglia projecting to the guinea-pig adrenal gland. It is interesting to note that no postganglionic projection arises from the coeliac ganglia in either rat or guinea-pig.

It appears that in both the rat and guinea-pig, the efferent and afferent cell bodies arise from closely related vertebral levels, T9–T10 in the rat (Kesse *et al.* 1988; Parker, Afework & Coupland, 1990) and T10 in the guinea-pig (Mohamed *et al.* 1988). This might indicate that a reflex pathway exists at these levels for control of adrenal secretion. This is in addition to a parasympathetic pathway found in both animals by Coupland, Parker, Kesse & Mohamed (1989). As to whether the reflex pathways control secretion directly or through adrenal blood flow remains to be ascertained. But owing to tracer spread within the adrenal gland, the efferent/afferent terminals may be present in both cortex and medulla; even though, and apart from the observations of Prentice & Wood (1975) using the false transmitter 5-hydroxydopamine, no evidence of adrenergic innervation of the adrenal medulla, at least in the rat, has been obtained (Coupland, unpublished). It is most likely that the preganglionic fibres project to the adrenal medulla and the postganglionic fibres to the cortex, a view that is supported by the morphological findings of Unsicker (1973) and Coupland (1972, 1984). Additionally, possible afferent-like terminals have been found in both cortex and medulla (Mohamed *et al.* 1988).

Further elucidation of these pathways must await anterograde tracer studies which are at present under way in this department.

SUMMARY

The pre- and postganglionic sympathetic innervation of the guinea-pig adrenal medulla was investigated using the retrograde neuronal tracers Fast Blue and WGA-HRP.

Labelled preganglionic cell bodies were located in the intermediolateral horn of spinal segments T3–L2, the majority (73·9%) were found between T6–T12 representing 70·2% of the total number of labelled cells; the segment T10 contained the largest number of labelled neurons.

Labelled postganglionic cell bodies were found in the paravertebral ganglia between vertebral levels T3–T12 (representing 22·6% of the total labelled neurons), the maximum number was found at T10. In addition, labelled neurons were found in the suprarenal ganglion (representing 7·2%). No labelled cells were found in the coeliac ganglia.

The labelled neurons were found ipsilateral to the site of injection into the left adrenal gland.

It is concluded that the guinea-pig adrenal gland receives both a pre- and a significant postganglionic sympathetic innervation. The destination of these nerve fibres within the adrenal gland has yet to be determined.

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