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TIME COURSE OF DEGENERATION OF SHORT AND LONG POSTGANGLIONIC SYMPATHETIC NERVE FIBRES AND EFFECT OF PENTOBARBITONE AND COLCHICINE ON DEGENERATION

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1 The time-course of degeneration of sympathetic nerves was investigated by measurement of the endogenous noradrenaline content of the rat vas deferens, submandibular gland and spleen following sympathectomy.

2 Extirpation of the hypogastric plexus, superior cervical ganglion and coeliac plexus under pentobarbitone anaesthesia caused 50% depletion of the noradrenaline content of the vas deferens, submandibular gland and spleen in approximately 16, 19 and 21 h, respectively.

3 Under pentobarbitone anaesthesia, proximal sympathectomy (i.e., close to the end organ) produced depletion of the noradrenaline content of the submandibular gland 8 h earlier than that caused by distal sympathectomy. Under ether anaesthesia, the time difference in obtaining the same degree of depletion after the two procedures of sympathectomy was only 2 hours.

4 Removal of the superior cervical ganglion under ether anaesthesia resulted in almost complete depletion of noradrenaline content of the submandibular gland in 17 h, whereas when a similar operation was performed under pentobarbitone anaesthesia, nearly 24 h were required for the same degree of depletion. Similarly, the noradrenaline content of the spleen was depleted 4 h earlier if the coeliac plexus was ablated under ether as compared to pentobarbitone anaesthesia.

5 Local application of colchicine (10 mg/ml, 30 min) to postganglionic sympathetic nerve axons had no effect on the noradrenaline content of the submandibular gland up to 24 hours. However, removal of the superior ganglion following colchicine application considerably slowed the depletion of the noradrenaline content of the submandibular gland (at 17 and 20 h after ganglionectomy, 10 and 20% depletion, respectively, in the experimental gland, as compared to 70 and 80%, respectively, in the control gland).

6 To explain the results, it is proposed that injury to the sympathetic nerves at the site of sectioning triggers a signal (messenger substance) which travels down to the nerve endings to produce degeneration. Thus, the length of the extrinsic nerve fibre influences the time course of degeneration by changing the rate of transport of the messenger substance, whereas pentobarbitone and colchicine alter the synthesis and/or transport of the messenger substance to modify the time-course of degeneration.

Introduction

Extirpation of the sympathetic neuronal cell body results in degeneration of its postganglionic nerve fibre innervating different effector organs. The time-course of degeneration of sympathetic nerves, as judged by the disappearance of the endogenous noradrenaline (NA), varies considerably from one organ to another. For example, the removal of the cat superior cervical ganglion caused complete depletion of NA content of the nictitating membrane in 36 h (Kirpekar, Cervoni & Furchgott, 1962), but crushing the postganglionic nerve fibre caused disappearance of NA from the cat spleen in 3 days (Kirpekar, Wakade & Prat, 1970). The reason for the variation in the time taken for degeneration of nerve endings after sympathectomy is not well understood. Bareggi, Dahlström & Häggendal (1974) have suggested that some vital substance is carried by axoplasmic flow from the cell body to the nerve endings to maintain

the normal function of the nerves. If its supply is interrupted near, as compared to further away from, the effector organ, the substance is exhausted earlier, causing more rapid degeneration. Another possibility is that injury to the neurone produces a substance which then travels to the rest of the neurone, causing degeneration of the entire terminal portion of the neurone. In the present study, attempts have been made to test the latter possibility by denervating different sympathetic neuroeffector organs of the rat having various lengths of extrinsic sympathetic nerves, and also sectioning the same sympathetic neurone at different levels from the effector organ in order to obtain some information about the relationship between the length of the postganglionic sympathetic axon and the time necessary for its degeneration. In the course of the experiments it was accidentally discovered that the nature of the anaesthetic agent in the surgical procedure influenced the time course of degeneration. Also, colchicine, a substance which interferes with axoplasmic flow (Dahlström, 1970), was tested for its effect on the rate of degeneration. In all experiments, depletion of the NA content of the effector organs was taken as an index of degeneration of nerve endings. A preliminary report of this work has been given to the Sixth International Congress of Pharmacology (Wakade, Mark & Rosenberg, 1975).

Methods

For the surgical procedures, male albino rats weighing from 300 to 400 g were anaesthetized either with pentobarbitone sodium (35 mg/kg i.p.) or with ether. In the case of ether anaesthesia, animals regained their righting reflex and consciousness in about 30 min after the surgery, whereas pentobarbitone-treated animals remained deeply sedated for several hours. No attempts were made to check body temperature of these animals.

Denervation of vas deferens

The lower portion of the abdomen was opened by a midline incision, under aseptic conditions. The distal portion of the colon was gently pulled out, in order to locate the hypogastric nerves. With an illuminating-magnifying lamp, the distal end of the right hypogastric nerve was traced as far as possible. In most cases an area of about 0.2 cm ahead of this distal end was considered to be the site of the hypogastric plexus (Wakade & Kirpekar, 1971). After location of the plexus, it was very gently lifted by a pair of fine forceps, carefully separated, and then removed from the surrounding tissue. Necessary precautions were taken to avoid cutting any of the major blood vessels of the internal sex organs. Animals were then allowed to recover, and right and left (control) vasa deferentia were removed at different time periods.

Denervation of salivary gland

In most cases the right superior cervical ganglion was removed, under aseptic conditions (distal sympathectomy). In some experiments postganglionic sympathetic nerves were cut near the submandibular gland to produce proximal sympathectomy. The major artery supplying the right submaxillary gland was gently exposed. With a Zeiss dissecting microscope, postganglionic sympathetic nerve fibres which run along the blood vessel were carefully separated and cut just before entering the gland. Care was taken not to disrupt the blood supply to the gland. All animals were then allowed to recover, and both salivary glands were removed after different time intervals.

Denervation of spleen

The middle portion of the abdomen was opened by a midline incision, under aseptic conditions. By the use of an illuminating-magnifying lamp, the coeliac plexus was located at the origin of the splenic artery from the aorta. The plexus was carefully separated from major blood vessels and then cut away. Animals were allowed to recover, and the spleen was removed at various time periods.

Application of colchicine

The postganglionic sympathetic nerve fibres innervating the right submaxillary gland were isolated as described above. A small cotton pledget (1–2 mm) was soaked in a solution of colchicine (10 mg/ml) and gently placed on the nerve fibre for 30 minutes. Then the pledget was removed, and the area was carefully cleaned with fresh 0.9% w/v NaCl solution (saline). After colchicine pretreatment, the right superior cervical ganglion was removed and the animal was allowed to recover. In a control experiment the same procedure was followed, except that colchicine was replaced by saline. All animals were killed at various times after ganglionectomy, and right and left submaxillary glands were removed for NA assay. In some experiments, postganglionic nerve fibres innervating the right and left submaxillary glands were separated as described above, and then cotton pledgets soaked in colchicine (10 mg/ml) and saline solution were placed on the right and left nerve fibres respectively, for 30 min, after which the pledgets were removed and nerve fibres were thoroughly cleaned with fresh saline solution. Animals were allowed to recover and were killed at various times after colchicine treatment. Both glands were removed for NA assay. In these

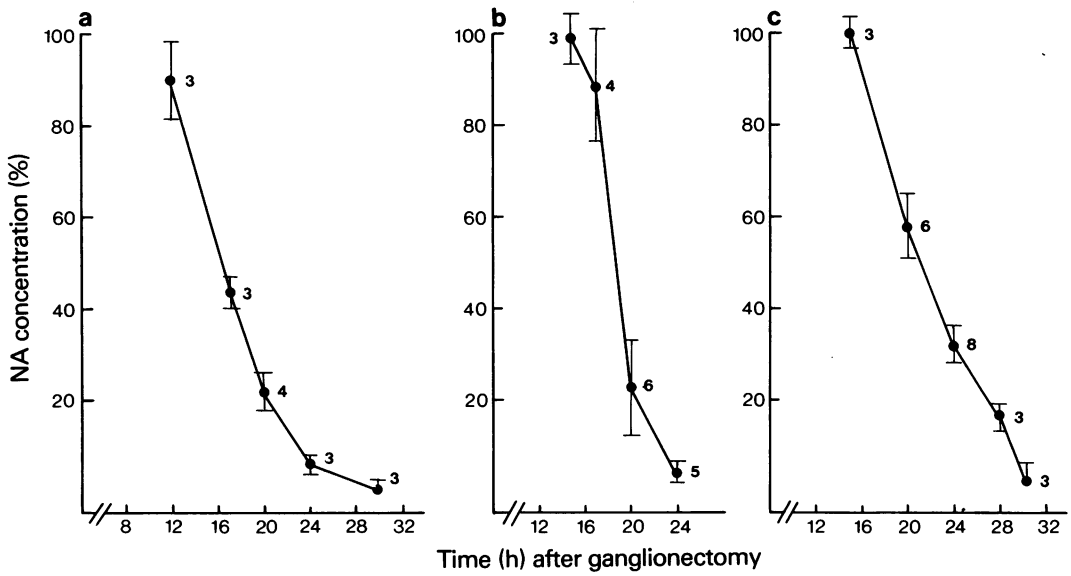

Figure 1 Noradrenaline (NA) content of the rat vas deferens, submandibular gland and spleen at various times after ganglionectomy. In the case of the vas deferens (a) and submandibular gland (b), NA concentration of the right tissue, measured at different times after removal of the hypogastric plexus and superior cervical ganglion, respectively, is expressed as a percentage of that found in the control left tissue. Spleen (c) was denervated by removing the coeliac plexus, and its NA content is expressed as a percentage of that found in normal spleen (0.50 ± 0.09, $n = 7$). Number of observations is shown beside each point. Vertical lines show s.e. mean.

experiments the mortality rate after colchicine treatment was less than 10%.

Removal of tissues

Rats were killed by a blow on the head, and appropriate sections of the body were opened to remove both submaxillary glands, spleen, or both vasa deferentia. The dissection of these tissues was carried out in oxygenated Krebs solution. In the case of the salivary gland, the sublingual gland which is located on the top portion of the submandibular gland and has practically no sympathetic innervation, was gently separated and then removed.

Extraction and analysis

Each tissue was rapidly blotted and weighed, and transferred to a 20 ml plastic tube containing 3 ml of ice-cold 0.4 N perchloric acid. Tissues were homogenized for 30 s with a Polytron homogenizer (Brinkmann Instruments), the homogenate was centrifuged and the supernatant was analyzed for NA by the method of Shellenberger & Gordon (1971). In all cases, standard solutions of NA were analyzed concurrently, with recovery ranging from 70 to 90%.

Drugs used

The drugs used were: (−)-noradrenaline bitartrate, colchicine (Sigma Chemical Company, St. Louis, Mo.); sodium pentobarbitone, Nembutal (Abbott Laboratories, Chicago, Ill.); ether anhydrous (Fisher Scientific, Fairlawn, N.J.).

Results

Effect of sympathectomy on noradrenaline content of the vas deferens, submandibular gland and spleen

In preliminary experiments it was found that the NA content of five control right and left vasa deferentia was 12.16 ± 1.17 and 11.68 ± 0.87 μg/gram. Similarly, there was no significant difference between NA content of the control right and left submandibular glands (2.13 ± 0.20 and 2.49 ± 0.23 μg/g, $n = 5$).

During the course of the present work it was noted that the NA content of the vasa deferentia and submandibular gland fluctuated considerably from one group of rats to another. For instance, the concentration of NA in the vas deferens ranged between 6.3 and 18.9 μg/g, and in the submandibular gland the

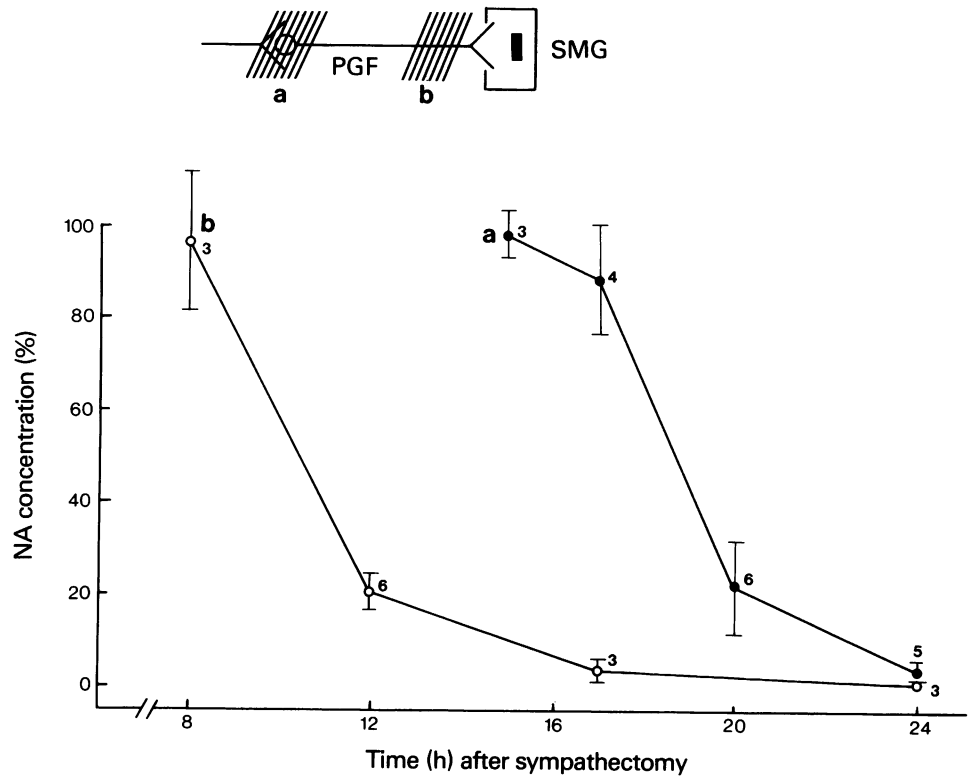

Figure 2 Effect of distal and proximal sympathectomy on noradrenaline (NA) content of the rat submandibular gland. Distal sympathectomy was achieved by removal of superior cervical ganglion (a, inset), whereas postganglionic nerve fibres (PGF) were cut close to the submandibular gland (SMG) to obtain proximal sympathectomy (b). NA content of SMG at various times after proximal (○) and distal (●) sympathectomy, is shown. Each value is expressed as a percentage of the contralateral control NA concentration. Number of observations is shown beside each point. Vertical lines show s.e. mean.

range was between 0.74 and 3.1 μg/gram. Despite such a marked variation from one animal to another, the NA concentration found in the right and left organ of the same animal was always comparable. Therefore, in each study NA values of the experimental organ were compared with the contralateral organs from the same animal. Figure 1 shows the effect of ablation of the hypogastric plexus, superior cervical ganglion and coeliac plexus on the NA content of the vas deferens, submandibular gland and spleen, respectively. In all cases, pentobarbitone was used as the anaesthetic agent during surgery on the rats. In three experiments, 12 h after ablation of the right hypogastric plexus there was no significant change in the NA content of the right vas deferens (9.96 ± 0.10 μg/g) as compared to the control left vas deferens (11.06 ± 1.02 μg/gram). After 17 h, the NA content was 4.96 ± 0.29 μg/g, and after 24 h, NA levels of the right vas deferens were markedly reduced to 0.61 ± 0.01 μg/gram. In the case of the submandibular gland, 17 h after removal of the right superior cervical ganglion the NA content of the right submandibular gland was not significantly altered. After 20 h, the NA content of the right gland was reduced to 0.88 ± 0.37 μg/g from a control value of 2.38 ± 0.38 μg/gram. Almost negligible levels of endogenous NA (0.10 ± 0.03 μg/g) remained 24 h after ganglionectomy. When the coeliac plexus was severed, NA content of the spleen was depleted from a control value of 0.50 ± 0.09 μg/g to 0.29 ± 0.03 μg/g in 20 hours. After 24 and 28 h it was further reduced to 0.16 ± 0.03 μg/g and 0.08 ± 0.06 μg/g, and complete loss occurred by 30 h after ganglionectomy.

From these initial studies it can be seen that the time required to produce 50% depletion of NA from the vas deferens, submandibular gland and spleen after sympathetic denervation was about 16, 19 and 21 h, respectively.

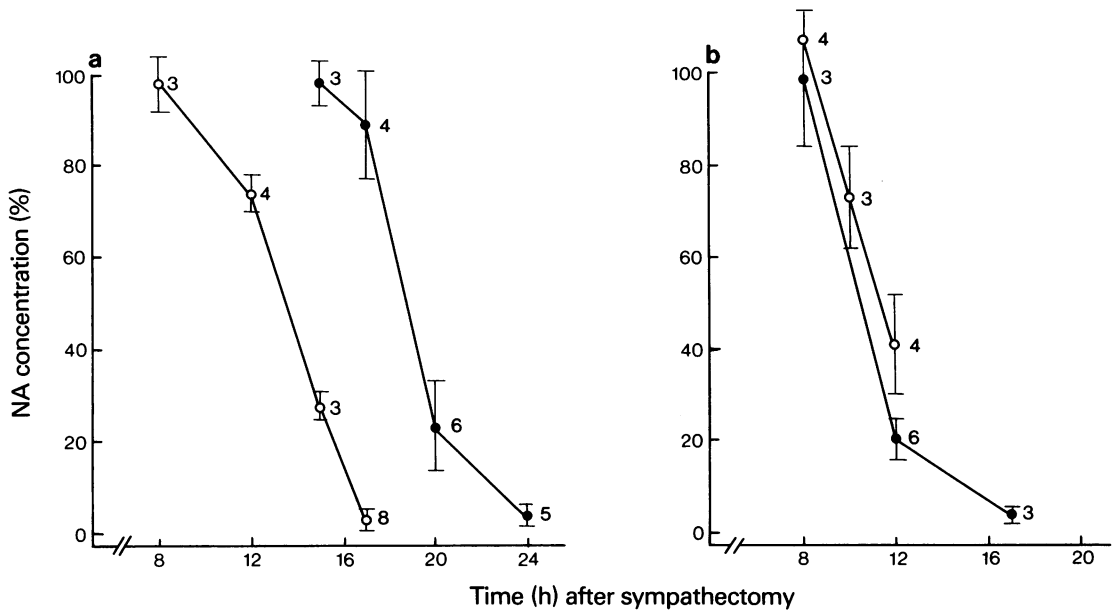

Figure 3 Effect of pentobarbitone and ether on the time-course of depletion of noradrenaline (NA) content of the rat submandibular gland after sympathectomy. (a) NA concentration of the gland at various times after removal of the superior cervical ganglion under ether (○) and pentobarbitone (●) anaesthesia. (b) NA concentrations of the gland at various times after cutting postganglionic nerve fibres near the submandibular gland under ether (○) and pentobarbitone (●). Number of observations shown beside each point. Vertical lines show s.e. mean.

Noradrenaline content of the submandibular gland after proximal and distal sympathectomy

As shown at the top of Figure 2, the submandibular gland of the rat was denervated either by removal of the superior cervical ganglion (a), or by cutting the postganglionic nerve fibre close to the gland (proximal sympathectomy (b)), under pentobarbitone anaesthesia. Eight hours after proximal sympathectomy there was no significant effect on the NA content of the right gland (curve b). However, in the next 4 h the NA content was reduced to 0.25 ± 0.04 μg/g as compared to the left control gland (1.25 ± 0.16 μg/gram). Practically complete loss had occurred by 17 h after the operation (0.11 ± 0.02 and 2.18 ± 0.10 μg/g). Curve (a) represents the mean NA values of the right submandibular gland at various times after removal of the right superior cervical ganglion (distal sympathectomy). These values are taken from Figure 1a. It is apparent from Figure 2 that there is about an 8 h delay in the onset of depletion of NA in the gland after distal sympathectomy as compared to proximal sympathectomy. Because of this difference, the NA content of the gland after proximal sympathectomy was depleted by more than 80% within 12 to 16 h, at which time the NA content of the gland after distal sympathectomy was not significantly altered.

Comparison of the effect of ether and pentobarbitone on the noradrenaline content of the submandibular gland after proximal and distal sympathectomy

The influence of pentobarbitone and ether (see Methods) on the depletion of the NA content of the submandibular gland after ganglionectomy and cutting of postganglionic nerve fibres is shown in Figure 3. The time required for the depletion of the NA content of the right salivary gland was drastically reduced (Fig. 3a, open circles) when the right superior cervical ganglion was removed under ether anaesthesia. Thus, 12 and 15 h after ganglionectomy, performed under ether, the NA content of the right gland was reduced to 0.86 ± 0.06 and 0.34 ± 0.01 μg/g from the control values of 1.27 ± 0.11 and 1.20 ± 0.2 μg/g, respectively. By 17 h there was virtually complete loss of endogenous NA content (0.17 ± 0.05 compared with 1.57 ± 0.24 μg/g). On the other hand, the NA content remained unaffected 15 to 17 h after ganglionectomy if pentobarbitone was used as an anaesthetic agent

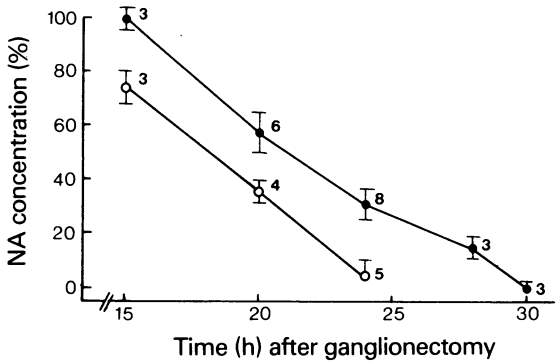

Figure 4 Effect of pentobarbitone and ether on the time-course of depletion of noradrenaline (NA) content of rat spleen after ganglionectomy. NA content of the spleen at various times after removal of coeliac ganglion under ether (○) and pentobarbitone (●) anaesthesia. NA concentration is expressed as a percentage of the control value (0.50 ± 0.09, $n = 7$). Number of observations is shown beside each point. Vertical lines show s.e. mean.

(solid circles). There was about a 6 h difference in the time required to obtain 50% depletion of the NA content of the submandibular gland denervated by ganglionectomy under ether and pentobarbitone. Figure 3b shows that the time needed to produce loss of NA at various times after cutting postganglionic nerve fibres near the gland was not significantly different whether the operation was carried out under ether or pentobarbitone anaesthesia.

Influence of ether and pentobarbitone on the time-course of depletion of spleen noradrenaline after sympathectomy

In another series of experiments spleens were denervated by removal of the coeliac ganglia from rats anaesthetized with either ether or pentobarbitone. When ether was used (Figure 4, open circles), the NA content of the spleen was reduced to 0.38 ± 0.04 μg, as compared to the control value of 0.51 ± 0.04 μg, 15 h after ganglionectomy. After 20 h the NA was depleted by over 60%, and almost undetectable levels (0.09 ± 0.02 μg/g) were reached by 24 hours. On the other hand, under pentobarbitone anaesthesia the NA content of the spleen was unaltered 15 h after ganglionectomy, 50% depletion had occurred by 22 h, and complete loss of endogenous NA content occurred only after 30 hours. Thus, in the case of the spleen, as with the salivary gland, pentobarbitone considerably slowed the process of degeneration of splenic nerves following ganglionectomy.

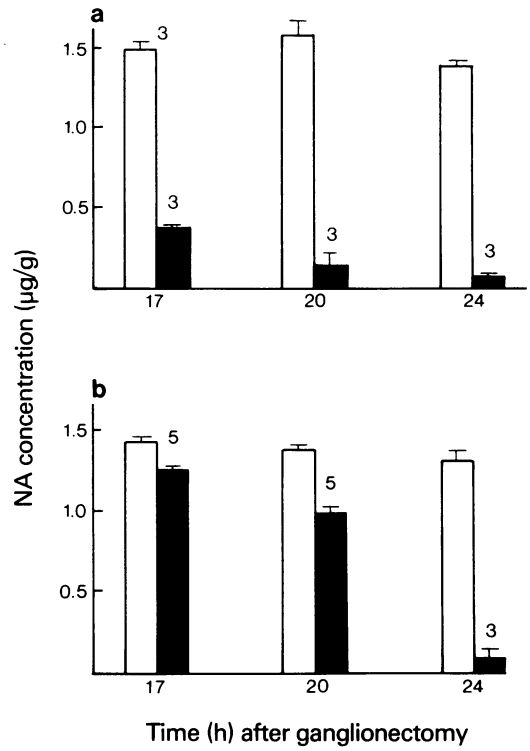

Figure 5 Effect of colchicine on depletion of noradrenaline (NA) content of the rat submandibular gland produced by ganglionectomy. NA concentration of the gland after application of saline (a) and colchicine (b) to postganglionic sympathetic nerve fibres of the gland for 30 min before removal of the superior cervical ganglion. Solid columns represent NA content of the right gland at various times after removal of the right superior cervical ganglion. Open columns represent NA content of the contralateral control gland. Number of observations is shown for each pair of columns. Vertical lines at top of columns show s.e. mean.

Influence of colchicine on the time-course of depletion of noradrenaline of the submandibular gland after ganglionectomy

The effect of local application of colchicine to postganglionic nerves was investigated on the depletion of NA in the submandibular gland caused by ganglionectomy. Rats were anaesthetized with ether, and the right sympathetic postganglionic nerve fibres, just before entering the submandibular gland, were exposed and soaked with a solution of colchicine (10 mg/ml) or saline for 30 minutes. Then the right superior cervical ganglion was removed, and the NA content of both glands was analyzed at various times

after ganglionectomy. These results are shown in Figure 5. In preliminary control experiments, when postganglionic nerve fibres were pretreated with saline rather than colchicine (Figure 5a) before removal of the ganglion, there was a loss of approximately 70% of NA from the salivary gland in 17 h, and of approximately 90% 20 h after ganglionectomy. When similar types of experiments were carried out with colchicine pretreatment of postganglionic nerve fibres, it was consistently found that such treatment prevented the loss of NA 17 h after ganglionectomy (Figure 5b). Even 20 h after colchicine pretreatment and removal of the superior cervical ganglion, the NA content of the salivary gland was reduced by only 25%. Only after 24 h was the extent of depletion in the colchicine-treated rats comparable to that in the controls.

Three experiments were performed to see if pentobarbitone and colchicine treatments would have any additive delaying effects on the degeneration of sympathetic nerve endings of the submandibular gland. In these experiments rats were anaesthetized with pentobarbitone, colchicine was applied to the right postganglionic nerves, and then the right superior cervical ganglion was removed, as described above. It was found that after removal of the ganglion, the NA content of the gland was reduced to about 70 and 90% in 20 and 24 h, respectively. These reductions did not differ significantly from those found at the same times when no pentobarbitone anaesthesia was used without colchicine treatment of the nerves (Figure 1a).

In order to study the effect of colchicine alone on the NA content of the submandibular gland, several experiments were carried out in which post-ganglionic nerves of the glands of rats under either anaesthetic were treated with colchicine as described above. As shown in Table 1, 17, 20 and 24 h after colchicine treatment the NA content of the right submandibular gland was not significantly different from that of the left gland, the sympathetic nerve fibres of which were treated with saline.

Table 1 Noradrenaline content of submandibular gland at various times after application of saline or colchicine to its postganglionic sympathetic nerve fibre

Time (h)	*Noradrenaline content (μg/g)* *Saline*	*Colchicine*
17	1.86 ± 0.29 (3)	1.79 ± 0.10 (3)
20	2.12 ± 0.17 (4)	2.00 ± 0.24 (3)
24	2.05 ± 0.27 (4)	1.90 ± 0.15 (4)

Right and left postganglionic nerve fibres were exposed, and treated with saline and colchicine (10 mg/ml), respectively, for 30 min; animals were allowed to recover, and submandibular glands were removed at various times after their application.

Discussion

It has been well established that after sympathectomy, several changes occur simultaneously in the terminal portion of the nerves. For example, the disappearance of the endogenous NA of the effector organ (von Euler & Purkhold, 1951) is accompanied by degeneration of postganglionic nerve terminals, as judged by electron micrograph studies (Van Orden, Bensch, Langer & Trendelenburg, 1967), and by impairment of neutronal uptake of exogenous NA (Hertting, Axelrod, Kopin & Whitby, 1961). Therefore, in the present study the time course of depletion of NA content of different sympathetic effector organs after sympathectomy was used as a measure of the time course of degeneration of the nerves.

It was found that the time course of degeneration of the postganglionic nerve fibres after sympathectomy was different for the vas deferens, submandibular gland and spleen of the rat. In the case of the vas deferens, 50% depletion of the NA occurred in 16 h, whereas about 19 and 21 h were needed to obtain the same degree of NA loss from the submandibular gland and spleen, respectively, after removal of their sympathetic ganglia (Figure 1). These observations can be explained by supposing that the length of the extrinsic postganglionic nerve fibre is an important factor in determining the time course of the degeneration of the sympathetic nerves (Kirpekar *et al.*, 1970). It is known that vasa deferentia of rat (Birmingham, 1970) and guinea-pig (Watanabe, 1969; Wakade & Kirpekar, 1971) are innervated by short postganglionic nerves arising from the hypogastric plexus located about 0.5 cm away from the target organ. On the other hand, postganglionic nerves originating in the superior cervical ganglion and the coeliac ganglion must travel at least 2 to 3 cm before innervating the submandibular gland and spleen, respectively.

That the length of the postganglionic sympathetic nerve plays a role in determining the time required for denervation was further supported by experiments in the submandibular gland. Under pentobarbitone anaesthesia, distal as against proximal sympathectomy resulted in prolongation of the degeneration process by about 8 hours.

The unexpected observation was that the time course of degeneration of the sympathetic nerves following removal of the sympathetic ganglion was dependent on the nature of the general anaesthetic agent used during surgery. When the superior cervical ganglion was removed from rats anaesthetized with

pentobarbitone, 24 h were needed for essentially complete degeneration of the sympathetic nerves of the submandibular gland. The same surgical procedure carried out under ether anaesthesia required only 17 h to achieve an equivalent degree of degeneration. Thus, the process of degeneration of the sympathetic nerve endings was delayed by about 8 h if ganglionectomy was performed under pentobarbitone instead of ether anaesthesia. The delaying effect of pentobarbitone on sympathetic degeneration was further confirmed by showing that degeneration of the sympathetic nerve terminals of the rat spleen after coeliac ganglionectomy required 4 additional hours to obtain the same degree of degeneration achieved under ether anaesthesia.

Distal, as compared to proximal, sympathectomy performed under ether anaesthesia led to prolongation of degeneration of sympathetic nerves of the submandibular gland by only 2 hours. As mentioned above, under pentobarbitone anaesthesia the time delay was 8 hours. Very recently, Almgren, Dahlström & Häggendal (1976) have reported that depletion of NA occurs 2 h earlier if sympathectomy is achieved by crushing the postganglionic nerves near the salivary gland, as compared to that caused by removal of the superior cervical ganglion. In the case of motor nerves, Harris & Thesleff (1972) observed that denervation is prolonged by 2 h for each cm of nerve stump remaining after axotomy.

Another finding was that prior application of colchicine to postganglionic nerve fibres prolonged the time for degeneration of sympathetic nerve endings caused by removal of the superior cervical ganglion under ether anaesthesia. The NA content of the submandibular gland was reduced by 75 and 95%, 17 and 24 h after ganglionectomy, respectively. However, application of colchicine to postganglionic nerve fibres before removal of the ganglion resulted in only 10 and 30% loss of NA in the same time periods. Earlier, Lundberg (1972) found that degeneration contraction of periorbital smooth muscle, seen after sympathetic denervation, was delayed by systemic administration of colchicine and vinca alkaloids. From the above observations it appears that the time course of sympathetic nerve degeneration following ganglionectomy is not only related to the length of the extrinsic nerve fibres, but can also be influenced by pharmacological agents.

It has been suggested that degeneration of the terminal portion of the sympathetic neurone after axotomy occurs as a result of lack of protective substance coming from the cell body to the terminals via axoplasmic transport (Bareggi *et al.*, 1974; Almgren *et al.*, 1976). The rate of sympathetic degeneration was comparable to that of the axoplasmic flow (8 mm/hour). Variations in the time course of degeneration of sympathetic effector organs having different lengths of extrinsic nerve fibre, as observed in the present study, can be explained on such a hypothesis. However, experiments in which colchicine slowed the process of degeneration after ganglionectomy (Figure 5), and failed to alter the NA content after its application to the nerve (Table 1), cannot be explained on the assumption that a protective substance is involved in the process of degeneration. Alternatively, I should like to propose another hypothesis to account for my observations in the present study. Removal of the sympathetic ganglion, or cutting postganglionic nerve fibres, triggers a positive signal (messenger of degeneration) which travels to the terminal portion of the neurone, causing its destruction. According to this concept, the longer the distance between the site of injury and the terminal portion of the nerves, the more time is required for the messenger to reach the terminals, and hence longer time for degenerative changes to occur in the effector organ. For this reason, the salivary gland is depleted of NA considerably earlier when the sympathetic nerves are cut proximally as compared to distally, and also the vas deferens loses its NA content earlier than the spleen following ganglionectomy. It may very well be that pentobarbitone or its metabolic product, in some unknown manner, slows down the synthesis and/or transport of the messenger substance from the site of injury to the terminal area of the sympathetic nerves. Whether any change in body temperature of the animal could account for slowing down of the degeneration procedure following pentobarbitone anaesthesia, is open to question. Some evidence for a messenger substance travelling down the nerve fibres from the site of injury comes from the experiments with colchicine. Since colchicine application prolonged the time for degeneration after ganglionectomy, it is reasonable to assume that the messenger substance was transported to the nerve endings via microtubules. Interference with the function of the microtubules by colchicine resulted in disruption of the axoplasmic flow of the messenger substance, and thereby protected the terminals from degeneration, at least for some time.

Mere blockade of the axoplasmic transport by a 30 min application of colchicine to the nerve fibre did not alter the NA content of the submandibular gland up to 24 h after its application although similar treatment drastically interfered with the degeneration of nerves caused by ganglionectomy. These findings can be taken to support the idea that a messenger substance is formed only after injury to the neurone, and then travels to the nerve endings via axoplasmic flow to produce degeneration.

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