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THE INTERNAL ANAL SPHINCTER IN THE CAT: A STUDY OF NERVOUS MECHANISMS AFFECTING TONE AND REFLEX ACTIVITY

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SUMMARY

1. Smooth muscle activities in rectum and internal anal sphincter have been recorded using intraluminal balloons.

2. Reflex activation of the sphincter, caused by distension of the rectum, has been assessed before and after various combinations of blocking drugs.

3. Responses to stimulation of hypogastric or sacral nerves, or to the administration of drugs with autonomic actions have been tested before and after various combinations of blocking drugs.

4. Results indicate that the tone of the internal anal sphincter is influenced by a number of neural mechanisms. These include motor pathways involving both α-adrenergic and cholinergic mechanisms and inhibitory pathways involving both β-adrenergic and non-adrenergic non-cholinergic mechanisms.

5. Cholinergic contractions of the sphincter were converted to relaxations after α-adrenergic blockade. This indicates that the contractions are an indirect effect operating through an adrenergic reflex. Cholinergic relaxations may also be indirect and operate through reflex inhibition secondary to rectal contractions.

6. Sphincteric motor activity is controlled largely through α-adrenergic mechanisms by adrenergic nerves acting directly on the muscle. β-Adrenergic inhibitory mechanisms are thought to operate indirectly via ganglia.

7. The over-all control of the sphincter is by complex reflex mechanisms involving numerous pathways and the activity of the sphincter at any one time is determined by the net balance between motor and inhibitory influences.

8. Sacral nerve stimulation indicated that it contains cholinergic nerves to the rectum, non-adrenergic non-cholinergic inhibitory axons to the sphincter and variable numbers of adrenergic axons to the sphincter.

9. Responses of the sphincter to drugs and nerve stimulation were often variable, as has been described many times in the literature. It is considered that this is due to complex combinations of indirect reflex effects, secondary to activation of structures outside the sphincter, operating with or against direct effects on the sphincter itself.

INTRODUCTION

The hindgut in the cat is made up of both smooth and striated muscle. The smooth muscle portion comprises the longitudinal and circular layers of the rectum, the internal anal sphincter and the two accessory muscles of defaecation, the ano-coccygeus and recto-coccygeus (Howard & Garrett, 1973). The external anal sphincter, on the other hand, is composed of striated muscle. Reflex activity occurs between all of these components to facilitate effective emptying of the rectum during defaecation and for the maintenance of continence at other times. The present investigation has been concerned with the nervous control of the smooth muscle of the hind-gut involved in such reflexes.

Morphologically the internal anal sphincter is derived from the circular muscle of the rectum but despite this continuity marked differences have been observed in the motor activity of the two structures. Langley & Anderson (1895) noted, for example, that whilst stimulation of lumbar sympathetic nerves in the cat caused relaxation of the rectum, the internal anal sphincter was contracted strongly. Different effects were also recorded during stimulation of sacral parasympathetic nerves. Similar results were reported from studies of hind-gut physiology in the cat by Garry (1932, 1933*a*, *b*).

In the present study, responses of the anus and rectum to the administration of drugs with autonomic actions and to direct nerve stimulation have been recorded by inserting air-filled balloons into the hindgut of anaesthetized cats. In addition, reflex activity between the rectum and the sphincter has been studied by distending the middle third of the rectum and recording the effects on anal canal pressures. The effects of autonomic blocking agents on the nervous pathways involved in these reflexes have also been examined.

METHODS

Cats of either sex, weighing from 2·5 to 4 kg were studied. Fifteen of the animals were anaesthetized with I.P. and I.V. pentobarbitone sodium (Nembutal 30 mg/kg), and two with I.V. chloralose (50–75 mg/kg). Eight animals were paralysed with an I.V. infusion of suxamethonium chloride (0·1 mg/kg.min) after the induction of anaesthesia with either a halothane-nitrous oxide mixture or chloralose. Paralysed animals were maintained on positive pressure ventilation via a cuffed endotracheal tube. Body temperature was monitored with an intra-oesophageal thermometer and maintained at 37° C by an electrically heated pad placed under the supine animal. Blood pressure was recorded from a femoral arterial cannula.

Hindgut intraluminal pressures were recorded with a probe that was a modification of the recording device described by Lawson & Nixon (1967). It consisted of a proximal air filled rectal balloon (diameter 2·5 cm) and a distal air filled cylindrical chamber, *ca.* 3 mm in diameter and 8 mm in length which was positioned within the lumen of the internal anal sphincter. This chamber was constructed from brass and covered with thin latex tubing. Rectal and sphincteric pressures were recorded synchronously via pressure transducers and displayed on a pen-recorder (San-Ei or Sanborn). The rectal balloon could be inflated or deflated with air without disturbing the sphincter recording device.

Bipolar or unipolar platinum electrodes were used to stimulate hypogastric and sacral nerves with 2 msec pulses of 4–6 V at a frequency of 20/sec. Initially drugs were given I.V. but in later experiments an intra-aortic cannula, inserted through a femoral artery, was preferred. Intra-aortic drug injection produced more rapid responses which frequently preceded changes in blood pressure or pulse rate. The abolition of cardiovascular responses to cholinergic and adrenergic drugs was used as evidence for the effectiveness of blocking agents.

The following drugs, dissolved in normal saline, were used: acetylcholine perchlorate (BDH Chemicals); acetyl-β-methyl choline chloride, DL-noradrenaline hydrochloride, DL-isoprenaline hydrochloride, dihydroergotamine tartrate, DL-propranolol hydrochloride, hexamethonium bromide (Sigma); DL-adrenaline acid tartrate (Evans Medical); atropine sulphate (Burroughs Wellcome).

RESULTS

In all experiments a record of normal anal canal activity was obtained after the induction of anaesthesia but before administration of drugs with autonomic actions.

Normal anal canal activity. The smooth muscle of the internal anal sphincter exhibited rhythmic contractions, the frequency varying between 12 and 36/min. This activity was often superimposed on a fluctuating base line pressure recorded as slow contractions with a duration of 10–20 sec. This contractile activity of smooth muscle was most clearly seen when the voluntary muscle of the external sphincter was paralysed in lightly anaesthetized animals with the depolarizing agent succinyl choline. Conversely, smooth muscle contractions were completely hidden by the stronger voluntary muscle activity of lightly anaesthetized animals that were not paralysed with striped muscle relaxants.

Reflex activity

Recto-anal reflexes. Rectal distension caused a reflex relaxation of the internal anal sphincter in twenty out of twenty-five cats (Fig. 1*a*). This reflex relaxation was most easily demonstrated in lightly anaesthetized cats paralysed with succinyl choline, and least impressive in animals under Nembutal anaesthesia. Deep Nembutal anaesthesia lowered the tone of the anal canal, perhaps through its side effects of atropine-like cholinergic blockade and sympathetic ganglion depression (Goodman & Gilman, 1965) so that inhibitory responses were sometimes difficult to detect.

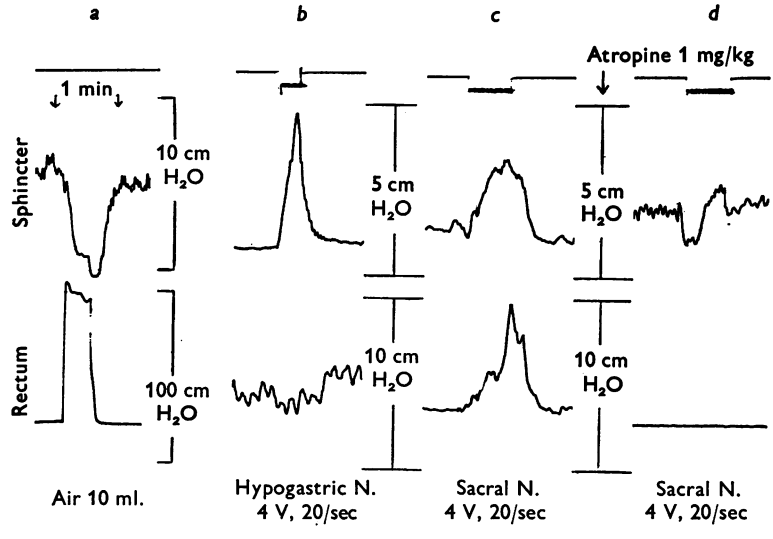

Fig. 1. Traces from the same cat showing intraluminal pressure responses in the sphincter and the rectum (pentobarbitone anaesthesia). *a*, distension of the rectal balloon by air caused reflex inhibition of the sphincter. *b*, hypogastric nerve stimulation caused contraction of the sphincter but little change in the rectum. *c*, sacral nerve stimulation caused contraction of both the sphincter and the rectum. *d*, after atropine sacral nerve stimulation caused no effect on the rectum but the sphincter response had become biphasic with an initial inhibition followed by a contraction.

The degree of sphincter relaxation was related to the distending volume of air in the rectum. Thus 5 ml. air might cause a slight fall in sphincter pressure whereas 15 ml. would often cause a sustained fall and complete inhibition of rhythmic activity. The speed of rectal inflation also affected the sphincter response. For example, a sphincter contraction might precede relaxation when the rectum was stretched rapidly whereas slow

rectal distension produced inhibition alone. Sphincter tone returned rapidly as the rectum deflated.

Vesico-anal reflexes. Compression of the urinary bladder and spontaneous voiding were accompanied by a strong contraction of the internal anal sphincter (Fig. 2). The bladder was therefore kept empty by needle

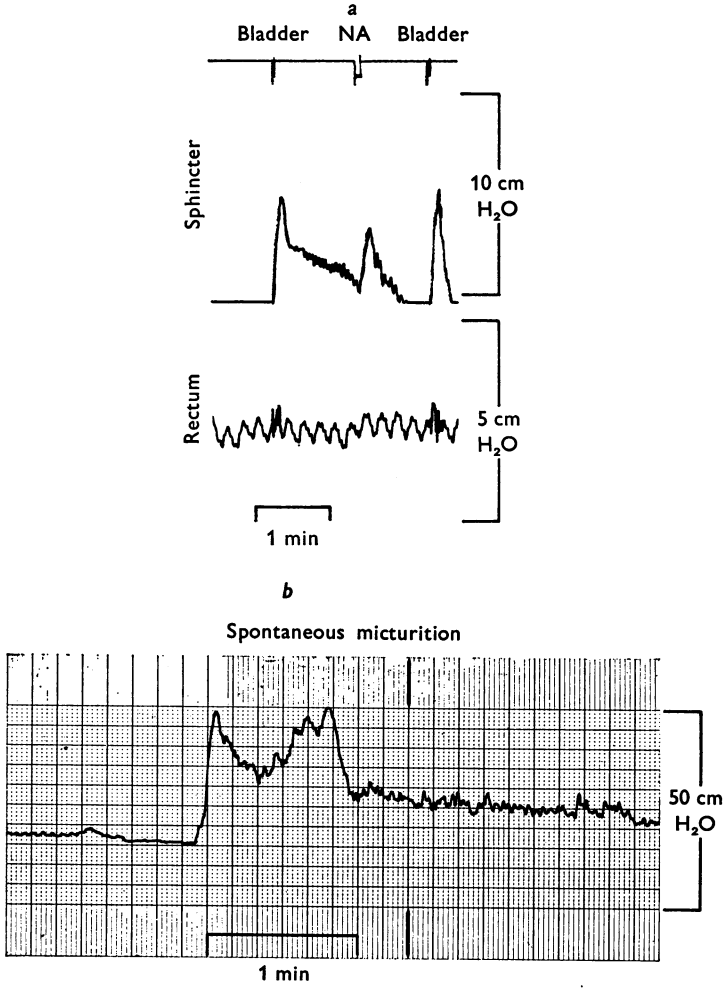

Fig. 2. Traces from different cats showing intraluminal pressure responses accompanying increased tension in the bladder. *a*, squeezing the bladder, 1st and 3rd mark, caused a contraction of the sphincter; the rectum trace was altered only by the effect of mechanical movement induced by this procedure. At the intermediate mark noradrenaline NA, 20 μg/kg I.V., caused a sphincter contraction without affecting the rectum (pentobarbitone anaesthesia). *b*, spontaneous micturition was accompanied by a vigorous contraction of the sphincter (succinyl choline).

aspiration throughout the experiments. It was noted that these responses were abolished by α-adrenergic blockade with dihydroergotamine (0·25–1·5 mg/kg).

Nerve stimulation

Hypogastric nerves. Division of the hypogastric nerves below the inferior mesenteric ganglion caused a temporary reduction in the tone of the internal anal sphincter whilst the spontaneous contractile activity of the rectum increased. Sphincteric tone tended to return after an interval of about 30 min. Electrical stimulation of the distal cut end of the nerves in fifteen experiments always caused a strong contraction of the sphincter (Fig. 1*b*). Rectal responses during hypogastric nerve stimulation were noted in four experiments. In three animals, the rectum relaxed whilst in one contractions were recorded each time the nerves were stimulated. α-Adrenergic blockade completely abolished sphincter contractions in all nine animals tested. In two experiments hypogastric nerve stimulation after α-block caused sphincter relaxation, a response abolished by the β-blocking drug propranolol (1–4 mg/kg). Neither atropine nor hexamethonium affected the contractile sphincteric responses to hypogastric nerve stimulation.

Sacral nerves. Section of the sacral nerves always caused a reduction in the tone of the anal canal which lasted throughout the experiment. Electrical stimulation of the distal cut ends always caused a contraction of the rectum (Fig. 1*c*). The effects on the internal anal sphincter, however, were not consistent. In two out of six experiments stimulation resulted in a rise in sphincter tone and enhancement of rhythmic activity (Fig. 1*c*). In three experiments relaxation was the constant response whilst in one experiment alternating contractions and relaxations were recorded. The rise in sphincter tone caused by sacral nerve stimulation was converted to a biphasic response by atropine in one experiment (Fig. 1*d*). It was unaffected by atropine in another experiment but was converted to an inhibitory response after the injection of dihydroergotamine. The inhibitory responses in three experiments were not affected by atropine, or β-blockade, nor were they abolished by hexamethonium infusion (100 μg/kg.min).

The action of drugs

Noradrenaline and adrenaline (1–10 μg/kg). These drugs caused sphincter contractions in eighteen out of nineteen experiments (Fig. 3). The force of contraction was dose related and post-contraction inhibition was a common event. In one experiment both drugs caused an initial sphincter relaxation which was succeeded by a contraction. Contractions of the sphincter were abolished by α-adrenergic blockade with dihydro-

ergotamine (0·25–1·5 mg/kg). Rectal tone and activity were reduced in two experiments by both adrenaline and noradrenaline (Fig. 3) but in four experiments the effect was stimulation blocked by dihydroergotamine.

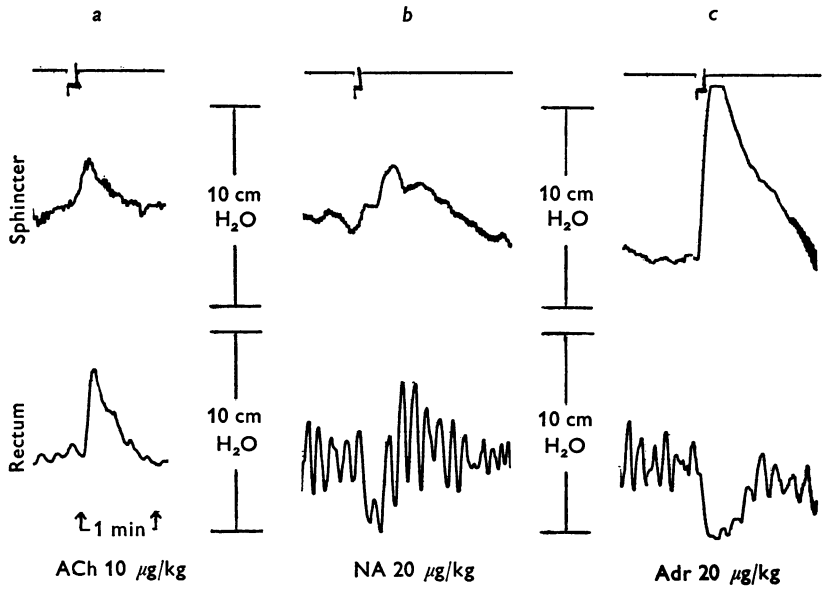

Fig. 3. Traces from the same cat showing rectal and sphincteric responses to drugs (pentobarbitone anaesthesia). *a*, acetylcholine, ACh 10 μg/kg I.V., caused a motor response in both rectum and sphincter. *b*, noradrenaline, NA, 20 μg/kg I.V., caused a motor response in the sphincter and a biphasic response in the rectum. *c*, adrenaline, Adr 20 μg/kg I.V., caused a strong motor response in the sphincter and an inhibitory response in the rectum.

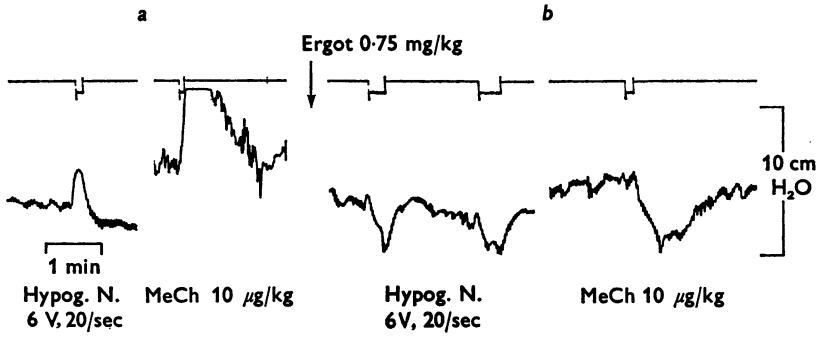

Fig. 4. Traces from the same cat showing sphincteric responses to hypogastric nerve stimulation and methyl choline I.V. (pentobarbitone anaesthesia). *a*, both caused sphincteric contraction in the absence of blocking drugs. *b*, after α-adrenergic blockade by dihydroergotamine, 0·75 mg/kg I.V., both now caused a relaxation of the sphincter.

Isoprenaline (1–10 μg/kg). Both rectal and sphincter muscle relaxed in all experiments. These effects were abolished by β-adrenergic blockade, using propranolol (1–4 mg/kg).

Acetylcholine and methacholine (2–10 μg/kg). Rectal contraction was a constant response (Fig. 3). Sphincter responses, however, were variable. In fifteen out of twenty experiments rectal contraction was accompanied by contraction and augmented rhythmic activity in the internal sphincter which lasted for up to 2 min (Fig. 3). A post-contraction inhibitory phase occurred frequently. In four experiments relaxation of the sphincter accompanied rectal contractions whilst in one animal small doses of ACh (< 5 μg/kg) caused relaxation of the sphincter and larger doses (> 5 mg/kg) caused contractions. Cholinergic drug effects were always abolished by atropine.

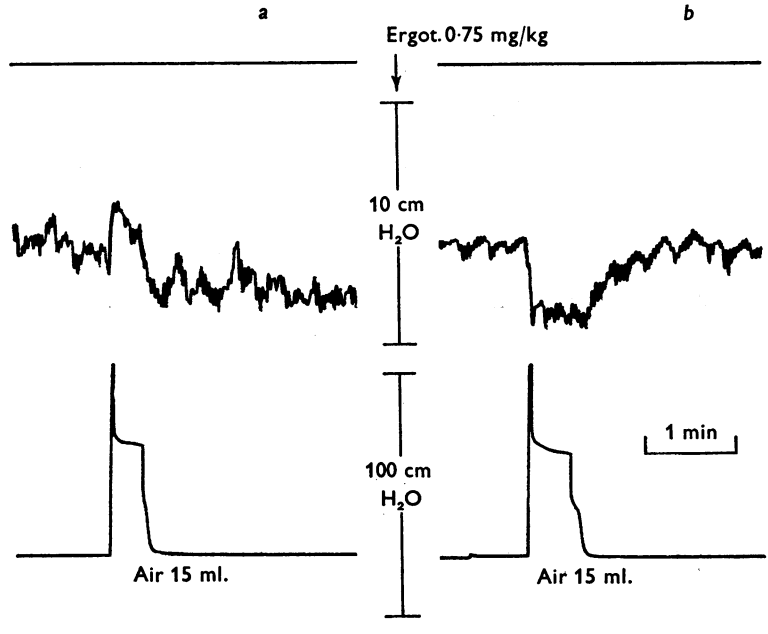

Fig. 5. Traces from the same cat showing responses in the sphincter (above) on distension of the rectal balloon with air (below) (pentobarbitone anaesthesia). *a*, rectal distension caused sphincteric contraction in the absence of blocking agents. *b*, after α-adrenergic blockade with dihydroergotamine, 0·75 mg/kg I.V., rectal distension now caused relaxation of the sphincter.

In each of five experiments the sphincter contraction response to the injection of cholinergic drugs was converted to a relaxation response by α-adrenergic blockade (Fig. 4). Cholinergic relaxation was subsequently abolished by atropinization.

Effects of autonomic blocking drugs

The recto-anal inhibitory reflexes elicited in twenty experiments were unaffected by α-adrenergic blocking with dihydroergotamine. However, α-blockade did abolish the small reflex contractions which sometimes preceded sphincter inhibition. In three animals, however, rectal distension caused sphincter contraction until the administration of an α-blocking agent, when the more usual recto-anal inhibition occurred (Fig. 5).

β-Blockade with propranolol (1–4 mg/kg), on the other hand, converted the reflex from inhibition to contraction in four experiments (Fig. 6). This contraction was abolished by dihydroergotamine (0·25–1·5 mg/kg), an inhibitory reflex always appearing after a combination of α- and

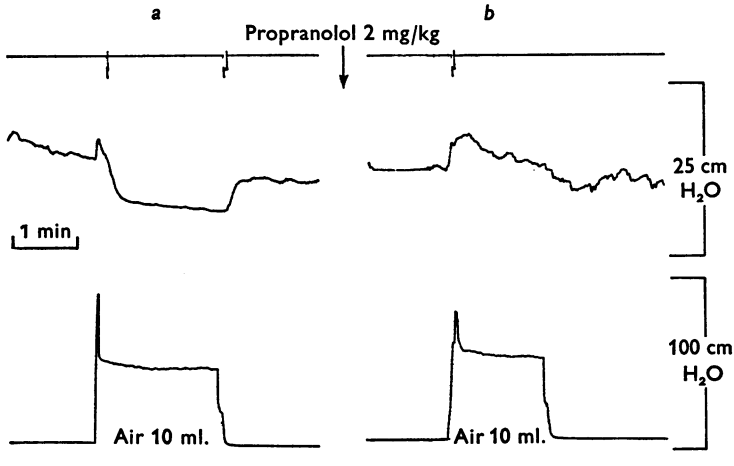

Fig. 6. Traces from the same cat showing responses in the sphincter (above) on distension of the rectal balloon with air (below) (pentobarbitone anaesthesia). *a*, rectal distension caused sphincteric inhibition preceded by a small motor response in the absence of blocking drugs. *b*, after β-adrenergic blockade by propranolol, 2 mg/kg, rectal distension now caused contraction of the sphincter.

β-blockade. Small doses of atropine ($< 0{\cdot}5$ mg/kg) were either without effect or slightly raised anal canal tone, but recto-anal inhibition persisted. Larger doses ($> 0{\cdot}5$ mg/kg) caused loss of sphincter tone which recovered slowly. Recto-anal inhibition could still be induced after the recovery of tone.

When a combination of α- and β-blockade was induced in combination with atropinization, a characteristic type of recto-anal reflex was recorded (Fig. 7). This reflex was characterized by a slow relaxation, a persistent reduction in tone after rectal deflation, and a slow recovery phase (seven experiments). This reflex could only be abolished by an infusion of a ganglion blocking agent (hexamethonium) (Fig. 7).

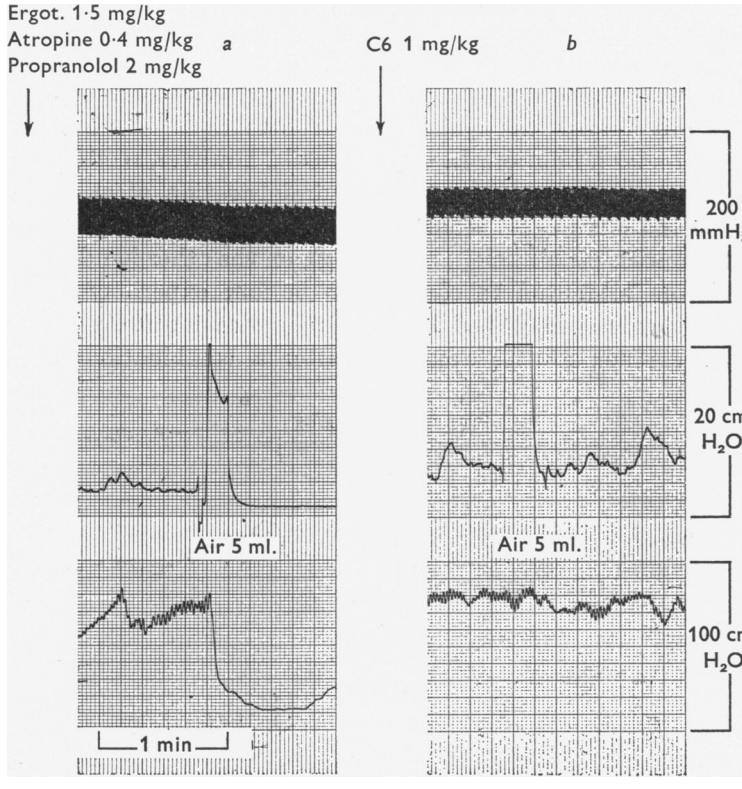

Fig. 7. Traces from the same cat showing responses of the sphincter to rectal distension after various blocking drugs (chloralose anaesthesia). Upper trace arterial pressure, middle trace rectal balloon, lower trace anal balloon. *a*, after dihydroergotamine, ergot., 1·5 mg/kg I.V., atropine 0·4 mg/kg I.V., and propranolol, 2·0 mg/kg I.V. (continued with 5 μg/kg.min I.V.), rectal distension caused an inhibition of the sphincter which slowly reached its peak and more slowly returned to the original level. *b*, after subsequent ganglionic blockade with hexamethonium, C6, 1 mg/kg I.V., distension of the rectum now caused no response in the sphincter.

DISCUSSION

The present investigation indicates that the tone of the internal anal sphincter of the cat is influenced by at least four nervous mechanisms. An adrenergic excitatory pathway has been demonstrated in the hypogastric nerves and, as division of these nerves resulted in a relaxation of the anal canal, it appears that there is normally a sympathetic impulse traffic which maintains sphincteric tone through action on α-receptors. In contrast the β-adrenergic drug isoprenaline always relaxed the

sphincter muscle. Relaxation also occurred in two experiments during hypogastric nerve stimulation after α-adrenergic blockade, which supports the idea that some of the inhibitory processes in the sphincter may be caused by sympathetic nerves acting through β-adrenergic mechanisms.

The variable effects of hypogastric nerve stimulation and noradrenaline on the rectum suggested a role for both α- and β-neuroeffector sites in the control of rectal activity. Recent histochemical studies have revealed a direct innervation of both longitudinal and circular muscle in the cat rectum (Howard & Garrett, 1973) and so provide a morphological basis for these results.

Contractions of the sphincter after the injection of cholinergic drugs were converted to relaxations by α-adrenergic blockade, the relaxation being abolished by atropine. This sequence suggests that the cholinergic-induced contractions are mediated through adrenergic reflexes. The contractile effects of acetylcholine on other organs, e.g. the bladder, and the reflex sympathetic activity and release of catecholamines, which may have been induced by the fall in blood pressure, could be responsible for these reflexes.

Stimulation of the sacral nerves produced contractions of the rectum but variable contractile and inhibitory responses in the sphincter. The rectal contractions were abolished by atropine. Contractions of the sphincter were abolished or converted to relaxations by α-adrenergic blockade. These findings suggest that the sacral nerves involve cholinergic motor mechanisms to the rectum and adrenergic pathways to the sphincter. Furthermore, the sphincter relaxation demonstrated after sacral nerve stimulation was unaffected by atropine and combined α- and β-adrenergic blockade, which suggests that the sacral nerves also involve non-cholinergic non-adrenergic inhibitory pathways. Infusions of hexamethonium did not affect the inhibitory responses caused by stimulation of distal cut ends of the sacral nerves, which suggests that a proportion of neurones on the inhibitory pathways lie proximal to the point of section and outside of the wall of the hind-gut. Previously, non-adrenergic non-cholinergic neurones have been thought to be restricted to the gut wall (Burnstock & Costa, 1973).

The results of simple drug injection and nerve stimulation suggest, therefore, that the tone of the internal anal sphincter and rectum is influenced by four nervous pathways involving α-adrenergic and cholinergic motor mechanisms, opposed by β-adrenergic and non-cholinergic non-adrenergic inhibitory mechanisms. Further, the poor responses elicited in sphincters with low tone suggest that normal relaxation is due principally to an inhibition of the α-adrenergic motor activity rather than a direct inhibition of the muscle.

What role do these nervous mechanisms play in reflex activity between the rectum and anal sphincter? During defaecation a bolus entering the caudal portion of the large bowel initiates a strong rectal contraction which is accompanied by a dilatation of the anal canal. In the present investigation a recto-anal inhibitory reflex was recorded after division of both sacral and hypogastric nerves and when the recto-anal canal was isolated but for the blood supply. Thus it would appear that some of the reflex pattern operates within intramural pathways, although this is not to say that such mechanisms are not normally influenced by extrinsic pathways.

β-Blockade either reduced or reversed the recto-anal inhibitory reflex. This is further evidence for the presence of a β-adrenergic inhibitory mechanism in sympathetic reflexes in the hind-gut. Recto-anal inhibition occurred if both α- and β-adrenergic receptors were blocked together, indicating a further type of inhibitory pathway which was modified but not abolished by atropine. This persistence of recto-anal inhibition suggests the presence of reflex activity mediated through a non-cholinergic non-adrenergic pathway which has been described elsewhere in the gastro-intestinal tract (see the review by Furness & Costa, 1973). Further, the abolition of this reflex mechanism by either the administration of the ganglion blocking agent hexamethonium or the isolation of the rectum below the distending balloon (Garry, 1933*b*) suggests that the reflex is associated with ganglia situated within the bowel wall. This may explain the results of previous investigations where an inhibitory reflex was recorded in spite of destruction of the cauda-equina and sacral nerve roots (Garry, 1933*b*; Denny-Brown & Robertson, 1935; Schuster, Hendrix & Mendelhoff, 1963).

It therefore appears that the nervous control of the internal anal sphincter in the cat is mediated through a complex of several pathways, and the responses may be influenced by each or any of these pathways. The activity recorded from the anal canal of the intact animals is determined by the net balance between motor and inhibitory influences.

Motor activity is controlled in large part through an α-adrenergic mechanism acting directly on the muscle. Thus, stimulation of the distal cut ends of the hypogastric nerves is unaffected by ganglion blocking agents. In contrast, direct nerve-induced β-adrenergic inhibitory activity on the sphincter muscle is weak, and has only been demonstrated in a few animals after α-blockade. Systemic administration of propranolol, on the other hand, raises anal canal tone and increases the amount of α-adrenergic motor activity. These findings suggest that adrenergic inhibitory pathways may be associated with ganglia, inside or outside of the gut wall, and act mainly through β-receptors.

Cholinergic effects on the sphincter were inhibitory when α-blockade was established. It is suggested that although there may be a small direct muscle effect, most of the inhibitory responses recorded after cholinergic drug administration were examples of recto-anal reflex inhibition consequent upon strong rectal contractions. In support of this, cholinergic blockade with atropine in doses less than 0·5 mg/kg caused only small rises in the tone of the anal canal. Large doses of atropine (greater than 0·5 mg/kg) caused a reduction in anal canal tone, perhaps due to a ganglion blocking effect (Steinberg & Hilton, 1966).

Non-cholinergic non-adrenergic inhibition was demonstrated in sacral nerve stimulation and in reflex activity between the rectum and anal sphincter. This latter activity could be blocked by a ganglion blocking agent, suggesting a neuronal pathway – perhaps within the myenteric plexus of the bowel wall. It is suggested that this is an important inhibitory mechanism in sphincter function.

Recent histochemical studies of the hind-gut in the cat (Garrett & Howard, 1972; Howard & Garrett, 1973) have revealed a very dense innervation of the internal anal sphincter by adrenergic and cholinesterase-positive nerves. The present physiological study suggests a motor role for the adrenergic nerves in the sphincter, but the role of cholinergic nerves remains a matter of debate.

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