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PREGANGLIONIC PARASYMPATHETIC VAGAL INNERVATION OF THE PYLORUS: A HRP STUDY IN THE FERRET
(MUSTELA PUTORIUS FURO)

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ABSTRACT

The Brainstem origins of vagal preganglionic parasympathetic neurons innervating the pylorus was studied in the ferret using the Horseradish peroxidase (HRP) neurohistochemical technique. A total of 12 adult male and female ferrets (8 experimental and 4 controls) were used for the study.

In each experimental ferret the pyloric canal and antrum were injected with 0.1 ml of 30% HRP in normal saline. The control ferrets were injected in a similar manner as the experimental ferret with normal saline. After 48-72 hours of post-injection survival, each ferret was perfused transcardially first with normal saline followed by a fixative containing 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1M phosphate buffer, pH 7.4 at room temperature and finally with 10% buffered sucrose at 4°C. Craniotomy was performed and the brainstem excised from the brain and spinal cord. Transverse serial frozen sections of the brainstem were taken and processed for HRP neurohistochemistry and were analyzed under light and dark-field microscopy.

The results of the study showed that in the experimental ferrets, neurons of the Dorsal motor nucleus of the vagus nerve (DMNV) as well as those of the nucleus ambiguous (nA) were labelled with HRP. The control ferrets did not reveal any HRP labelled neurons.

Key Words: pylorus; vagal nuclei; horseradish peroxidase; ferret

INTRODUCTION

Whereas the pylorus is often regarded anatomically as an integral part of the stomach, it is functionally and histologically distinct from the proximal part of the stomach (1, 2). Several studies have been carried out on the pylorus in man and other species (3, 4, 5, 6).

Following these studies, it is now well established that the pylorus serves as an effective anatomical and physiological sphincter between the stomach and the duodenum. The role of the pylorus in the complex control of gastric emptying and the prevention of duodenogastric reflux has generated a lot of interest amongst investigators, in view of the impact of this function on the course and prognosis of gastroduodenal diseases (4, 5).

To date, there are limited studies on the origins of vagal neurons supplying the pylorus (6,7, 8, 9, 10, 11, 12). Furthermore, in the continued search for suitable animal models for human experimental studies, recent studies have shown that the ferret has very close resemblance to man with respect to its gross gastric anatomy (13, 14), gastric innervation (15, 16, 17), vagal regulation of gastric motility (18) and gastric acid secretions in response to various stimuli (19, 20). In spite of these revelations on the ferret and its increasing popularity as an experimental model for human studies, we do not know the origins of vagal fibers innervating the pyloric segment of the gastrointestinal tract in the ferret. The present paper reports the vagal innervation of the pylorus in the ferret using the HRP neurohistochemical technique.

MATERIALS AND METHODS

A total of 12 male and female adult ferrets weighing between 800gms and 1500gms were used for the study. All the ferrets were kept in a well-ventilated and illuminated animal house. Each ferret was kept in a cage to which was fitted a water bottle with a special dispenser which allows the ferret free access to water. They were all fed with special ferret cubes.

For surgical exposure of the pylorus, each ferret was anaesthetized with an intraperitoneal injection of sodium pentobarbitone (Sagatal, May & Baker Limited, Dagenham) in a dose of 60mg/kg-body weight. A midline laparotomy was then performed and the pylorus along with the stomach were exteriorized.
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EVALUATION OF TRINIDAD AND TOBAGO TRADITIONAL MEDICINE I: EFFECTS OF TRADITIONAL FOLK REMEDY ON DIARRHOEA.

N V Offiah¹, S Ali Hosein², R Joseph³, S Maharaj³, S Mohammed¹, F Sirica-Ramsewak²

ABSTRACT

Hot aqueous extract of the bark of *Anacardium occidentale* (Cashew), commonly used in Trinidadian folk medicine for the treatment of diarrhoea was evaluated for antidiarrhoeal activity. The extract inhibited castor oil-induced induced diarrhoea in rats as judged by a decrease in the number of wet faeces in the extract-treated rats. The extract also inhibited the propulsive movement of intestinal contents in mice. The extract showed no direct effect on the isolated guinea-pig ileum, however, it inhibited in a dose-related manner the contractile effects of acetylcholine, histamine, and 5-hydroxytryptamine. The inhibitory effects on these agonists were non-competitive in nature. Phytochemical tests revealed the main constituents as tannin, steroids, triterpenoid and carbohydrates. The results indicate that action of *A. occidentale* bark extract could be through a combination of inhibition of elevated transmitter released and reduced propulsive movement of the small intestine. There is merit in the folk medicinal use of the extract.

INTRODUCTION

*Anacardium occidentale* Linn (*Anacardiaceae*) is an evergreen tree found in countries geographically located between the Tropics of Cancer and Capricorn (1). The tree has gnarled spreading branches and the fruit is kidney shaped. In Trinidad, it is found in abundance in the central and northern areas of the Island. Natives consume both the fruit and nut as a delicacy while some "brew" the bark of the tree as tea for the treatment of diarrhoea. Other uses of the plant include the use of the leaves and fruits for arthritis, colds, and steam therapy for malaria (2), and the use of a strong decoction of the tree bark alone or with Malomay (*Euphorbia hirta*) for diarrhoea and dysentery (3,4). Also, the crude extract of *A. occidentale* is used traditionally in Nigeria for the treatment of infectious and septic diseases in both humans and animals (5).

With no scientific report on the antidiarrhoeal activity of the plant, it was therefore decided to evaluate the antidiarrhoeal activity of the hot aqueous extract of the bark of *A. occidentale*.

MATERIALS AND METHODS

Plant material: Fresh bark of *Anacardium occidentale* (Cashew) was collected from the University of the West Indies (U.W.I.) Field Station, Mount Hope, Trinidad. The identity of the plant (TRIN 19601) was established by Mrs. Yasmin Comeau, curator of the National Herbarium U.W.I., St. Augustine, Trinidad. Voucher specimens documenting this collection are deposited in the National Herbarium, UWI, St. Augustine, Trinidad. After collection, the fresh bark was chipped into small pieces.

Preparation of extract: A hot aqueous extract of the bark of this plant was obtained by boiling 73.2 g of chipped bark in 300 mL of distilled water for 20 min. After cooling at room temperature and filtering, the dried solid content of the extract was determined to be 41.3 mg/mL. The extract was used fresh or stored at -20°C with no preservative until needed.

Phytochemical tests: The freshly prepared extract of *A. occidentale* was analyzed by the method of Trease and Evans (6).

Animals used: The animals used for the experiments were Sprague-Dawley rats weighing between 180 and 250 g, albino mice (20 to 25 g) and guinea pigs (250 to 400 g) of either sex. The animals were bred and maintained at the animal house, School of Veterinary Medicine, Faculty of Medical Sciences, EWMSC, Mount Hope, Trinidad. The animals were maintained at uniform laboratory conditions in standard steel cages and were provided with standard commercial livestock feed and water *ad libitum*. The guinea pigs were also given green grass. All studies were carried out using six animals in each group.

Acute toxicity test: The intraperitoneal LD₅₀ of the extract in mice was determined using the method of Lorke (7).