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Intracerebroventricular neuropeptide Y stimulates bile secretion via a vagal mechanism

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Abstract

The effect of intracerebroventricular injection of neuropeptide Y on biliary secretion was studied in conscious dogs, prepared with gastric and duodenal fistulas and cerebroventricular guides. Bile secretion was increased in a dose-dependent fashion by intracerebroventricular neuropeptide Y. The peak increase was seen after 500 pM/kg of neuropeptide Y which resulted in a 30·2% increase in bile flow over the period 30–150 minutes after injection. (Control: 23·2 (1·2) ml/2 hours; neuropeptide Y 500 pM/kg: 30·5 (1·1) ml/2 hours). Biliary lipid composition was not altered significantly but bicarbonate output was increased at all doses tested. Intravenous infusion of neuropeptide Y (1000 pM) for 1 hour had no significant effect. Intracerebroventricular neuropeptide Y (1000 pM/250–300 mg body weight) also increased bile flow in urethane-anaesthetised rats. This effect was abolished by cervical vagotomy. The demonstration of a central stimulation of alkaline bile flow suggests that bile secretion may be subject to central modulation.

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Neuropeptide Y is a 36 amino acid polypeptide that was first isolated from the porcine brain in 1982.[1] It has been shown to be the most abundant neuropeptide in the central nervous system and is also widely distributed throughout the peripheral nervous system.[2] Neuropeptide Y has also been isolated from the gastrointestinal tract with large concentrations found in the biliary tree.[3] Because of its extensive tissue localisation, its effects on a variety of biological systems has been assessed. Central administration of neuropeptide Y has been shown to affect diverse physiological functions and behaviours including cardiorespiratory, neuroendocrine, and reproductive control, and regulation of circadian rhythms, memory, and feeding behaviour.[4 5] Indeed, neuropeptide Y is the most potent central stimulant of feeding known.[6] In addition, intracerebroventricular injection of neuropeptide Y increases gastric acid and pancreatic secretion in dogs, and it has been suggested that it may mediate the cephalic phase secretory response to feeding.[7]

Despite the fact that the liver has an extensive neural network, the importance of nervous regulation of hepatic function is poorly understood. In particular, the capacity of central nervous system centres to influence liver function has not been evaluated. However, the alterations in biliary secretion and composition that result from ablation of the peripheral innervation of the liver suggest that central neural stimuli may indeed modulate biliary secretion. The cephalic phase response to feeding is one of the best recognised examples of modulation of the gut secretory function by the central nervous system. Secretion of bile is not usually considered to be of much importance in this response since the gall bladder provides a mechanism for rapid delivery of bile into the duodenum at mealtime. Nonetheless, some studies have suggested that there is an increase in bile secretion as part of the cephalic phase.[8 9]

Since neuropeptide Y has been proposed as a mediator of the cephalic phase response this study was undertaken to examine the effect of intracerebroventricular administration of neuropeptide Y on bile flow in dogs. Additional studies of the effect of vagotomy on neuropeptide Y induced changes in bile flow were performed in urethane anaesthetised rats.

Methods

PREPARATION OF EXPERIMENTAL DOG MODEL

Four healthy adult female mongrel dogs weighing 18–20 kg were housed in thermoregulated (23°C) cages (8×8×5 ft) with 12 hour light–dark cycles. The dogs were fed canned dog food and had regular access to laboratory canine chow and water. Dogs were deprived of food but not water for 18 hours before surgery and experiments.

The approval of the Animal Welfare Committee at the Durham VA Medical Center was obtained before all studies and care of animals conformed to the guidelines set out in the *Guide for the care and use of laboratory animals*.[10]

After the induction of anaesthesia with sodium pentobarbital (50 mg/kg), a cerebroventricular guide was placed on the superior aspect of the skull to allow injection into the lateral ventricle as previously described.[11] Through a midline abdominal incision, a cholecystectomy was performed and the lesser pancreatic duct was ligated. Thomas cannulas were then inserted into the duodenum, opposite the biliary papilla, and in a dependent part of the stomach and brought out through the anterior abdominal wall. The animals were allowed a two week recovery period after surgery before experiments began.

EFFECT OF INTRACEREBROVENTRICULAR NEUROPEPTIDE Y INJECTION ON BILE FLOW IN THE DOG

These experiments were performed on conscious dogs supported by Pavlov harnesses. The duodenal cannula was opened and the common bile duct cannulated with a 6 FG ureteric catheter via the ampulla of Vater. Bile was then collected

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continuously in 15 minute samples. Gastric acid was diverted into a large bore collection tube via the opened gastric cannula. A intravenous infusion of 0·9% saline was then begun and after one hour was changed to an infusion of sodium taurocholate, 500 mg/hour (Calbiochem, La Jolla, CA, USA), which was maintained for the duration of the experiment. This replaced bile acid loss from the enterohepatic circulation. Bile flow was allowed to stabilise for two hours before intracerebroventricular injection of neuropeptide Y or control was given.

Intracerebroventricular injection was performed by passing a 1·5 inch spinal needle through the cerebroventricular guide. The appearance of clear cerebrospinal fluid in the needle hub confirmed correct placement of the needle in the lateral cerebral ventricle. All injections were given as a 200 μl bolus of neuropeptide Y (Peninsula, Belmont, CA, USA) dissolved in sterile 0·9% saline containing 0·1% dog albumin (Sigma, St Louis, MO, USA) or the carrier alone as control. After injection, bile was collected for a further three hours. Each experiment was performed in duplicate on each dog. In addition, the biliary response to peripheral neuropeptide Y was assessed by monitoring the effect of intravenous infusion of 1000 pM/kg/hour neuropeptide Y on bile flow for one hour. The animals were studied only twice weekly with a minimum interval of 48 hours between experiments.

To eliminate a local stimulatory effect of gastric acid on bile flow, dogs were pretreated with omeprazole 20 mg twice daily (Prilosec, MSD, West Point PA, USA) on the day before study. The effectiveness of gastric acid suppression was tested in an independent study on the same dogs. Gastric acid was collected at 15 minute intervals for one hour before and one hour after an intravenous injection of 6 μg/kg of pentagastrin (Sigma, St Louis, MO, USA).

EFFECT OF VAGOTOMY ON BILIARY RESPONSE TO INTRACEREBROVENTRICULAR INJECTION OF NEUROPEPTIDE Y IN THE RAT

Male rats (250–300 g) were obtained from Charles River Laboratories (Wilmington, MA, USA). After the induction of anaesthesia (urethane: 1·25 g/kg given intraperitoneally), ligation of the pylorus was performed, and a cannula inserted into the stomach to divert gastric secretion. A femoral vein and the common bile duct were cannulated with PE-50 tubing. A cervical vagotomy was performed through a midline neck incision and a tracheostomy tube was inserted at the same time. Bile was collected at 15 minute intervals. Bile salt loss was replaced by an infusion of 0·1 mg/kg/minute of sodium taurocholate dissolved in 0·9% saline via the femoral line. After collecting baseline samples for two hours, injection of neuropeptide Y (1000 pM in 10 μl 0·1% albumin in saline) or control (10 μl 0·1% albumin in saline) into the lateral ventricle was performed using a stereotactic instrument (David Kopf Instruments, Tijunga, CA, USA) (coordinates: AP, 1·5 mm; Lat, 2 mm; DV, 3·4 mm). Bile flow was collected for two hours after intraventricular injection. Four experimental groups were studied: neuropeptide Y alone, neuropeptide Y and vagotomy, control injection alone, and control injection and vagotomy. The correct placement of the intracerebroventricular injection was confirmed by injection of dye at the same coordinates after completion of study and subsequent localisation of dye in the ventricle on brain sectioning.

BIOCHEMICAL ANALYSES

Bile acid concentrations were determined by an enzymatic assay using 3-alpha-hydroxysteroid dehydrogenase with sodium taurocholate as the standard.[12] Phospholipid concentrations were determined by enzymatic reaction with phospholipase D (Wako, Osaka, Japan) and subsequent determination of free choline using colorimetry.[13] Total cholesterol content of bile was determined by quantitative enzymatic analysis by the method of Allain *et al*, using cholesterol reagent supplied by Sigma.[14] Gastric acid and biliary bicarbonate concentration were determined by titration and back-titration respectively to pH 7·0 using 0·1 M NaOH.

STATISTICAL ANALYSIS

Statistical comparison was by one way analysis of variance followed by comparison between groups by Fisher's least significant difference in all experiments. In experiments performed on dogs, each animal served as its own control. All

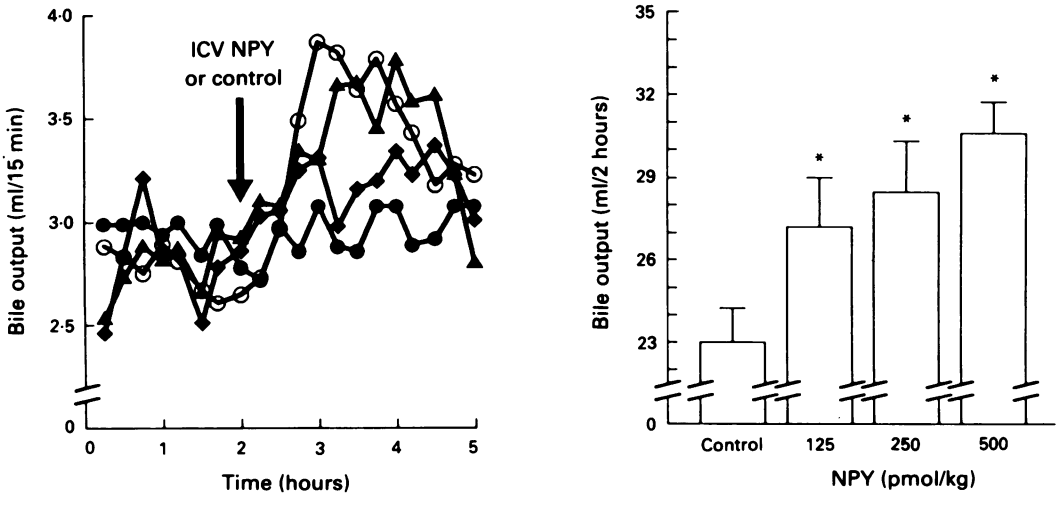

*Figure 1: Effect of 500 pM (○–○), 250 pM (▲–▲), 125 pM (◆–◆) intracerebroventricular (ICV) neuropeptide Y (NPY) and control (●–●) on bile flow in the dog. Each point represents the mean of eight studies (two experiments/dog). The bargraph shows the cumulative output of bile over two hours after ICV injection (+SEM) (*p<0·05 compared with control).*

Biliary lipid and bicarbonate output before and after intracerebroventricular of neuropeptide Y (NPY) injection in dogs (values, mean (SEM))

Dose of NPY (pmol/kg)	Bicarbonate (mmol/15 min)		Phospholipid (mmol/15 min)		Bile acids (mmol/15 min)		Cholesterol (mmol/15 min)	
	Before	After	Before	After	Before	After	Before	After
0 (Control)	158 (47)	163 (54)	71 (8)	55 (9)	284 (32)	250 (22)	2·3 (0·6)	1·8 (0·5)
125	127 (50)	188 (54)*	74 (13)	54 (7)	280 (8)	266 (4)	2·4 (0·4)	1·6 (0·3)
250	147 (58)	180 (58)*	62 (8)	49 (8)	259 (37)	209 (17)	2·4 (0·2)	1·3 (0·2)
500	175 (57)	206 (64)*	66 (12)	57 (5)	224 (20)	229 (10)	2·6 (0·3)	2·0 (0·1)

*p<0·05 compared with control.

values are expressed as mean (SEM). A p value of less than 0·05 was considered significant.

Results

INHIBITION OF GASTRIC ACID SECRETION BY OMEPRAZOLE

Omeprazole effectively suppressed the gastric acid response to pentagastrin. The mean (SEM) gastric acid outputs the hour before and the hour after pentagastrin administration were 0·54 (0·14) mmol/15 minutes and 0·59 (0·09) mmol/15 minutes respectively.

EFFECT OF NEUROPEPTIDE Y ON BILE FLOW IN DOG

Intracerebroventricular neuropeptide Y caused a dose-dependent increase in hepatic bile secretion. At the highest dose examined (500 pM/kg), it resulted in a 30·2% increase in bile output over the period 30–150 minutes after injection when compared with control (control: 23·2 (1·2) ml/2 hours; neuropeptide Y: 30·6 (1·1) ml/2 hours) (Fig 1). Cholesterol, bile acids, and phospholipid output concentrations were not significantly altered. The biliary bicarbonate concentration increased significantly in all doses examined but not in a dose-dependent fashion (Table).

Intravenous infusion of neuropeptide Y (1000 pM/kg) for one hour did not significantly affect bile flow (before infusion: 10·87 ml/hour, during neuropeptide Y infusion: 10·33 ml/hour). No significant changes in biliary bicarbonate or lipid composition were observed during peripheral infusion of neuropeptide Y.

EFFECT OF VAGOTOMY ON NEUROPEPTIDE Y STIMULATED BILE FLOW IN THE RAT

In the rat, after injection of control, bile output was 1·36 (0·008) ml/90 minutes for the period 30 to 120 minutes after injection (Fig 2). After injection of neuropeptide Y (1000 pmol/rat), bile output over the same time period was 1·54 (0·23) ml/90 minutes, which represents a 13·2% increase in bile flow (p<0·05). Bilateral cervical vagotomy abolished this response (Fig 2). Bile composition (bile acids, cholesterol, and phospholipids) was unaffected.

Discussion

This study shows a central stimulatory effect of neuropeptide Y on bile secretion that is mediated by the vagus nerve. The observation of a less noticeable stimulatory response to neuropeptide Y in the rat than in the dog may be explained by the fact that these rats were anaesthetised with urethane which is known to cause release of somatostatin – a known universal inhibitor of gut secretions.[15] A further explanation could be that rats have a small ductular component of bile secretion which may have been the primary target of the observed response, as evidenced by the increase in biliary bicarbonate output observed in the canine studies. Intravenous infusion of neuropeptide Y did not affect bile secretion suggesting that the choleretic effect of intracerebroventricular neuropeptide Y was not the result of peripheral leakage of the peptide across the blood brain barrier. In addition, the effect was not secondary to secretin release caused by duodenal acidification as this was avoided by pretreatment with omeprazole and diversion of gastric secretions.

Despite the fact that there have been many reports describing the central effects of neuropeptides on gastric and pancreatic secretion, there has been only one previous report of the effects of a centrally acting peptide on biliary secretion. In that study, Yao *et al* described the inhibitory effect of intracerebroventricular bombesin on biliary flow and bicarbonate secretion in the rat.[16] Several earlier studies had suggested the importance of vagal innervation in modulating bile formation. Tanturi and Ivy provided the first convincing evidence for the choleretic action of the vagus by showing that stimulation of the distal end of the divided vagus caused increased secretion of bile.[9] In addition, if the contralateral vagus were also divided, stimulation of the proximal end of the divided vagus

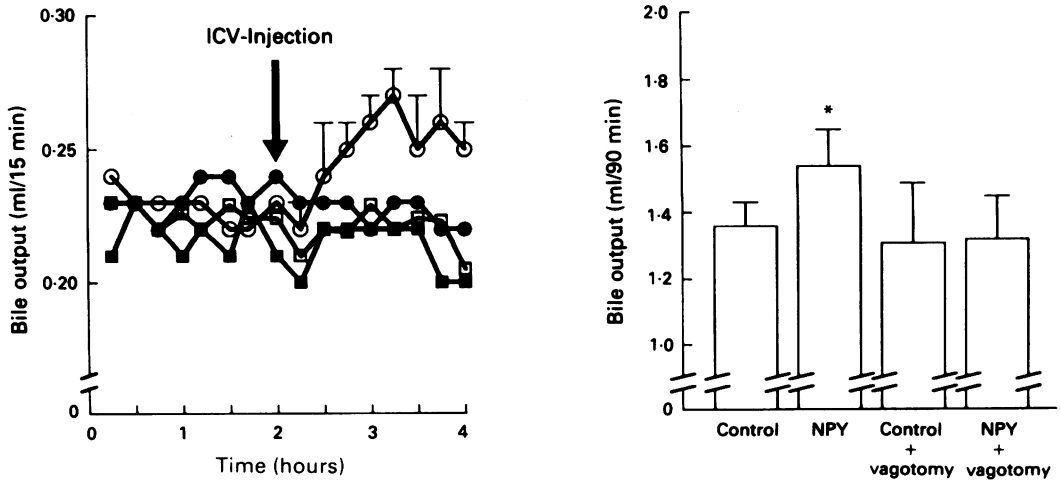

*Figure 2: Effect of 1000 pM intracerebroventricular (ICV) neuropeptide Y (NPY) (○–○) on bile flow+SEM, compared with control (●–●), 1000 pM ICV NPY and vagotomy (□–□), and control and vagotomy (■–■) in rats (n=9). The bargraph shows the cumulative output of bile over 90 minutes after ICV injection (*p<0·05 compared with control)*

caused a decrease in bile flow. These pioneering studies suggested that biliary secretion might be regulated by both vagal and non-vagal neural mechanisms. More recent studies have shown that hepatic denervation causes significant changes in the biliary lipid composition.[17] Despite these and other reports the role of central neural regulation of hepatic secretory function remained poorly defined.

Centrally administered neuropeptide Y caused a dose-dependent increase in bile flow associated with an increase in biliary bicarbonate output. Although the increase in biliary bicarbonate was observed at all intracerebroventricular doses of neuropeptide Y, no dose-related response of bilary bicarbonate was shown, presumably because of the inherent variability in normal bile bicarbonate concentration.

Neuropeptide Y has also been reported to increase substantially gastric acid and pancreatic exocrine secretion.[18] In the case of pancreatic secretion, stimulation of bicarbonate but not protein output was observed. This apparently selective effect on alkaline pancreatic secretion is similar to the specific effect of intracerebroventricular neuropeptide Y on the biliary bicarbonate observed in these studies. Alkaline bile secretion is known to contribute significantly to duodenal protection against acid injury to a similar (if not greater) degree than pancreatic secretion and stimulation of biliary bicarbonate secretion during or before feeding would seem to be of physiological benefit.

The finding by Fritz and Brooks that vagotomy abolishes the choleretic response to feeding in cholecystectomised dogs suggests that, at least as regards the digestive function of bile, neural regulation of biliary secretion may be of greater functional importance than is generally acknowledged. Powell demonstrated, in dogs with oesophageal fistulas, that sham feeding causes a hydrocholeresis, showing that there is a cephalic phase of bile secretion, albeit a relatively small one.[19] That the choleretic response to feeding is abolished by vagotomy suggests that this secretory event may possibly be mediated by a long vago-vagal reflex (similar to the increased production of gastric acid secondary to gastric distension), or that it may be a true cephalic phase event. If the choleretic response to feeding is mediated by a vago-bagal reflex, then it is likely that it relays in the solitary tract nucleus in the brainstem, which is the first relay nucleus for all afferent visceral sensation.[20] This nucleus contains neuropeptide Y receptors and it is potentially one site at which neuropeptide Y given intraventricularily may have bound to produce the effects observed in these studies.[21] Alternatively, neuropeptide Y may have produced its choleretic effect by binding at a hypothalamic level, where neuropeptide Y receptors are also plentiful.

However, a direct vasomotor effect of central neuropeptide Y on the hepatic artery vasculature, resulting in increased blood flow, cannot be ruled out entirely and may indeed be the mechanism causing the observed effect on biliary secretion in dogs where the arterial component to blood flow is significant as opposed to rats.

Because of its effects on feeding, and gastric and pancreatic secretion, it has been suggested that neuropeptide Y is a mediator of the cephalic phase secretory response to feeding. The site of neuropeptide Y's action on feeding seems to be in the hypothalamus, and it is likely that the cephalic phase response is also integrated at this level.[21] The effect on bile secretion seen after intracerebroventricular injection of neuropeptide Y in these studies may therefore be the result of neuropeptide Y binding to receptors responsible for releasing cephalic phase secretion.

Although nerve fibres are abundant in the liver, particularly in the portal triads, their metabolic importance remains obscure. Central nervous system regulation of hepatic function may provide a mechanism by which bile production is integrated with other metabolic activities particularly, digestive functions. Central neuropeptide Y release may be the primary stimulus for an integrated feeding response that includes the secretion of alkaline bile.

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