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α_2-Adrenergic regulation of galanin and norepinephrine release from canine pancreas

ANTON J. W. SCHEURINK, THOMAS O. MUNDINGER, BETH E. DUNNING,
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Scheurink, Anton J. W., Thomas O. Mundinger, Beth E. Dunning, Richard C. Veith, and Gerald J. Taborsky, Jr. α_2-Adrenergic regulation of galanin and norepinephrine release from canine pancreas. *Am. J. Physiol.* 262 (*Regulatory Integrative Comp. Physiol.* 31): R819–R825, 1992.—We found previously that electrical stimulation of the mixed autonomic pancreatic nerves (MPNS) in anesthesized dogs elicits marked and rapid increases of pancreatic output of both norepinephrine (NE) and galanin, and on that basis hypothesized a role for galanin as a sympathetic cotransmitter in the endocrine pancreas. In the present study, direct evidence was sought for the co-release of galanin and NE from canine pancreas by determining whether pancreatic galanin output is subject to modulation by presynaptic α_2-adrenergic mechanisms as has been established for NE. During MPNS (8 Hz, 1 ms, 10 mA, 10 min) in anesthesized dogs, both pancreatic NE and galanin outflow were increased with similar temporal patterns during consecutive stimulations. Blockade of presynaptic α_2-adrenoceptors with yohimbine increased and stimulation of presynaptic α_2-adrenoceptors with clonidine reduced NE and galanin outflow. Over all experiments, pancreatic spillover of galanin was highly correlated with that of NE. It is concluded that presynaptic α_2-adrenergic mechanisms modulate not only NE but also pancreatic galanin release, suggesting that galanin is co-released with NE from noradrenergic nerves in the endocrine pancreas.

adrenoceptors; sympathetic nervous system; yohimbine; clonidine; co-release; presynaptic regulatory mechanisms

ACTIVATION of the sympathetic nervous system leads to an increase of glucagon release and suppression of insulin secretion (3, 28, 31). It is generally assumed that these pancreatic responses to sympathetic activation are mediated by the classical neurotransmitter norepinephrine (NE). However, this assumption has been challenged (3, 8), and evidence for a role of neuropeptides in mediating certain of these effects on pancreatic islet function has started to accumulate (10). The 29-amino acid peptide galanin is the major candidate for this function for several reasons. First, galanin exerts sympathomimetic effects in the endocrine pancreas (7, 19). Second, galanin-like immunoreactivity (GLIR) is present in fibers innervating canine islets (1, 7). Third, recent experiments (9, 11) showed that sympathetic nerve stimulation leads to galanin outflow in quantities sufficient to account for the neural inhibition of insulin and partly for the neural stimulation of glucagon release.

The outflow of neurotransmitters in the sympathetic nervous system is modulated by presynaptic adrenergic mechanisms. Activation of presynaptic α_2-adrenoceptors inhibits, whereas stimulation of β_2-adrenoceptors facilitates, sympathetically induced NE outflow (5, 17, 23, 26, 29). Consequently, administration of the α_2-selective adrenoceptor antagonists markedly increased NE

outflow in several species, including dog (27, 30, 33). Blockade of presynaptic α_2-adrenoceptors also increased the outflow of neuropeptide Y, a peptidergic cotransmitter with NE in the sympathetic nerves in the dog (20, 21, 30). These data suggest that the release of both the main sympathetic neurotransmitter NE as well as the peptidergic cotransmitters in the sympathetic nervous system is modulated by presynaptic adrenergic regulatory mechanisms.

If galanin functions as a sympathetic transmitter with NE in the endocrine pancreas, then modulation of NE release by presynaptic adrenergic regulatory mechanisms should produce parallel effects on galanin outflow. The present study therefore investigates the influence of presynaptic adrenergic regulatory mechanisms on the outflow of galanin and NE induced by activation of the sympathetic nerves to the endocrine pancreas. Pancreatic galanin and NE spillover are measured in situ during stimulation of the pancreatic sympathetic nerves with and without administration of the α_2-selective agonist clonidine or the antagonist yohimbine in the halothane-anesthetized dog. Similar patterns of release of NE and galanin will support the hypothesis that NE and galanin are colocalized and co-released from sympathetic nerves of the endocrine pancreas.

MATERIALS AND METHODS

Animals and surgical procedures. Anesthesia was induced in overnight-fasted adult dogs of mixed breed (25–42 kg) by an intravenous bolus of an ultra-short-acting barbiturate, thiamylal (Surital, 20 mg/kg; Parke-Davis, Morris Plains, NJ). Animals were then intubated and ventilated with halothane (0.6–0.9% in 100% O_2) for maintenance of surgical anesthesia. After cannulation of a femoral artery and vein for blood pressure recording, blood sampling, and intravenous drug infusion, a midline laparotomy was performed to expose the duodenum and adjacent lobe of the pancreas. An extracorporeal shunt was introduced between the superior pancreatic duodenal vein (SPDV) and the portal vein, which contained a port for sampling of pancreatic venous blood and an electromagnetic flow probe for continuous monitoring of pancreatic venous blood flow. The mixed autonomic pancreatic nerves that course in the sheath of connective tissue surrounding the superior pancreatic duodenal artery (SPDA) were isolated immediately before their entrance into the pancreatic parenchyma and placed in a bipolar electrode (Harvard Apparatus, South Natick, MA). A 1-h recovery period followed these surgical procedures before experimentation.

Experimental procedure. Activation of the mixed autonomic pancreatic nerves was achieved by electrical stimulation (8 Hz, 1 ms, 10 mA, 10 min) of the sheath of connective tissue surrounding the SPDA. Blood samples for determination of NE were taken immediately before ($t = 0$ min) and twice during

nerve stimulation ($t = 5$ and 10 min). Blood samples for determination of GLIR were taken before ($t = -5$ and 0 min), three times during ($t = 2.5$, 5, and 10 min), and at 5 and 15 min after ($t = 15$ and 25 min) nerve stimulation. Within an experiment, a waiting period of at least 35 min occurred between consecutive nerve stimulations.

Blood sampling and chemical determinations. Blood samples were obtained simultaneously from the femoral artery and SPDV and were immediately placed on ice in tubes containing glutathione and ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) for NE measurements and a mixture of proteolytic enzyme inhibitors (6) for GLIR determination. Plasma NE was measured by a single isotope enzymatic method (24), and GLIR concentrations were determined by radioimmunoassay using synthetic porcine galanin standards and a non-COOH-terminally directed antiserum raised in rabbits against synthetic porcine galanin linked to bovine thyroglobulin (9, 11). Samples were centrifuged (4°C × 20 min), and plasma was stored at −80 (NE) or −20°C (GLIR).

Data analysis and statistics. Pancreatic outflow of NE was calculated according to the following formula that included a 75% extraction of arterial NE by the pancreas (2, 9): NE output = $([NE]_{SPDV} - 0.25[NE]_{FA}) \times (1 - \text{hematocrit}) \times \text{blood flow}_{SPDV}$. Pancreatic galanin outflow was calculated according to a modified version of this formula that corrected for 65% extraction of galanin by the pancreas and for the void volume of galanin in basal GLIR (9): GLIR output = $([GLIR]_{SPDV} - [GLIR]_{basal\ FA} - 0.65([GLIR]_{FA} - [GLIR]_{basal\ FA}) \times (1 - \text{hematocrit}) \times (\text{blood flow})_{SPDV}$. $[GLIR]_{basal\ FA}$ was defined as the mean of the FA levels at $t = -10$ min before the first nerve stimulation. Data were expressed as means ± SE. Wilcoxon matched pairs-signed rank tests were used to compare the levels of galanin and NE within an experiment with the baseline values at $t = 0$ min. Two-way analysis of variance and the Mann-Whitney U test were applied to determine significant differences between the results of an experiment and the data in the control experiment (*experiments 2* and *3*). Three-way analysis of variance was used to test differences in outflow between consecutive nerve stimulations in the control experiment. The criterion of significance was set at $P < 0.05$.

EXPERIMENTS AND RESULTS

Experiment 1: control experiment. The aim of the control experiment was to determine the effect of three consecutive stimulations of the mixed autonomic pancreatic nerve on the outflow of pancreatic NE and galanin. The results are presented in Figs. 1 and 2. Baseline outflow of GLIR and NE was 0.11 ± 0.04 and 3.2 ± 0.8 pmol/min, respectively, and did not change significantly between consecutive stimulations. Stimulation of the mixed pancreatic nerve led to an increased outflow of both GLIR and NE during all three nerve stimulations. Maximal outflow rates were 0.63 ± 0.08 pmol/min for GLIR and 90.7 ± 24.5 pmol/min for NE, both at $t = 5$ min during the first nerve stimulation. GLIR and NE outflow rates were significantly lower during the second and third nerve stimulation in comparison with the first one (respectively, maximal 0.40 ± 0.10 at $t = 10$ min and 0.35 ± 0.19 pmol/min at $t = 5$ min for GLIR and 58.6 ± 16.6 at $t = 5$ min and 45.2 ± 7.2 pmol/min at $t = 10$ min for NE).

Plasma levels of GLIR and NE in the femoral artery were 0.06 ± 0.01 and 1.03 ± 0.21 pmol/ml, respectively, immediately before the first nerve stimulation ($t = 0$) and did not change during or after the nerve stimulations.

Experiment 2: effects of α_2-adrenergic antagonism. The aim of the second experiment was to determine whether potentiation of pancreatic NE spillover via blockade of α_2-adrenoceptors was accompanied by enhanced pancreatic galanin outflow. The α_2-selective adrenoceptor antagonist yohimbine (50 μg·kg^{-1}·min^{-1}) was administered intravenously for 10 min starting 15 min before the second nerve stimulation. The results are presented in Table 1 and in Figs. 3 and 4. In this second experiment, the first stimulation of the mixed pancreatic autonomic nerve increased the outflow of GLIR and NE (baseline GLIR 0.15 ± 0.03 and NE 3.6 ± 1.3 pmol/min vs. stimu-
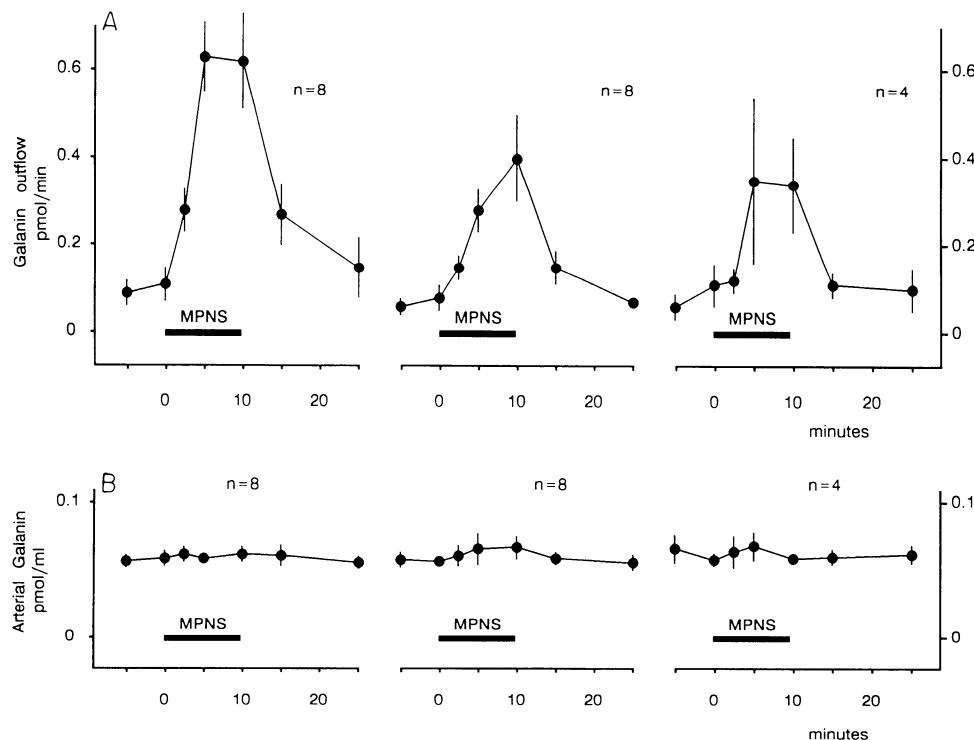

Fig. 1. Effect of 3 consecutive mixed pancreatic nerve stimulations (MPNS) on pancreatic outflow of galanin-like immunoreactivity (*A*) and arterial galanin levels (*B*). Data are averages ± SE. Nerve stimulation is indicated by horizontal bar at the bottom of each graph.
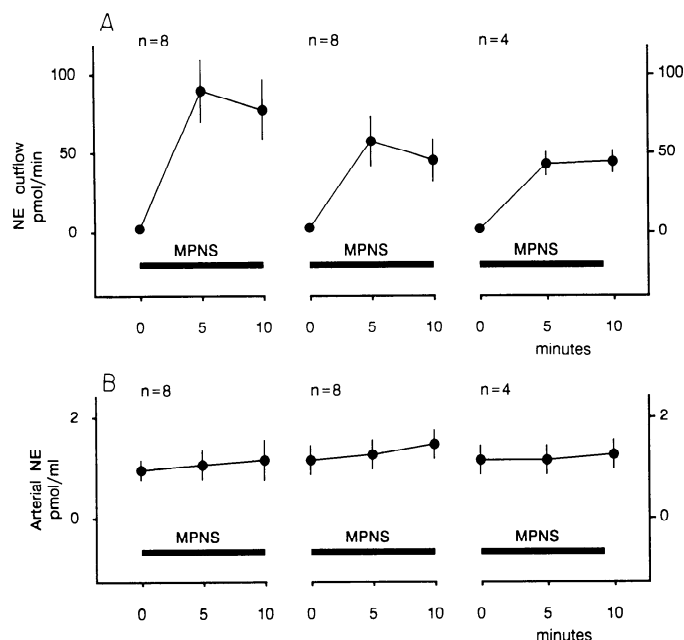
Fig. 2. Effect of 3 consecutive mixed pancreatic nerve stimulations (MPNS) on pancreatic outflow of norepinephrine (NE) (*A*) and arterial NE levels (*B*). Data are expressed as in Fig. 1.

Table 1. *Baseline outflow of GLIR and NE before and after treatment with α_2-selective adrenoceptor antagonist yohimbine (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$, n = 6) and α_2-selective adrenoceptor agonist clonidine (20 $\mu g/kg$, n = 4)*

	GLIR Outflow, pmol/min	NE Outflow, pmol/min
Experiment 2		
Control	0.15±0.03	3.6±1.3
Yohimbine	0.29±0.07	13.1±4.5
Experiment 3		
Control 1	0.11±0.04	3.2±0.8
Control 2	0.08±0.03	3.9±1.0
Clonidine	0.10±0.05	0.8±0.2

Values are means ± SE. GLIR, galanin-like immunoreactivity; NE, norepinephrine.

lated GLIR 0.69 ± 0.10 and NE 82.6 ± 8.7 pmol/min at t = 5 min). Thus the GLIR and NE outflow responses to the first nerve stimulation in *experiment 2* were almost identical to the results obtained during the first nerve stimulation in *experiment 1*. Administration of yohimbine caused an increase in both baseline (see Table 1) and stimulated outflow of GLIR and NE, with maximal responses of 1.25 ± 0.27 (GLIR) and 135.8 ± 20.7 pmol/min (NE) at, respectively, t = 2.5 and 5 min during nerve stimulation. The yohimbine-induced increases in transmitter spillover were significant at t = −5, 0, 2.5, 5, and 10 min for galanin, and at t = 5 and 10 min for NE in comparison with the data obtained during the second nerve stimulation in the control experiment. Administration of yohimbine also significantly increased plasma levels of galanin and NE in the femoral artery. Plasma galanin levels increased from 0.08 ± 0.01 to 0.13 ± 0.03 pmol/ml, and femoral NE plasma levels increased from 1.07 ± 0.18 to 3.43 ± 0.62 pmol/ml (data from t = 5 min during nerve stimulation).
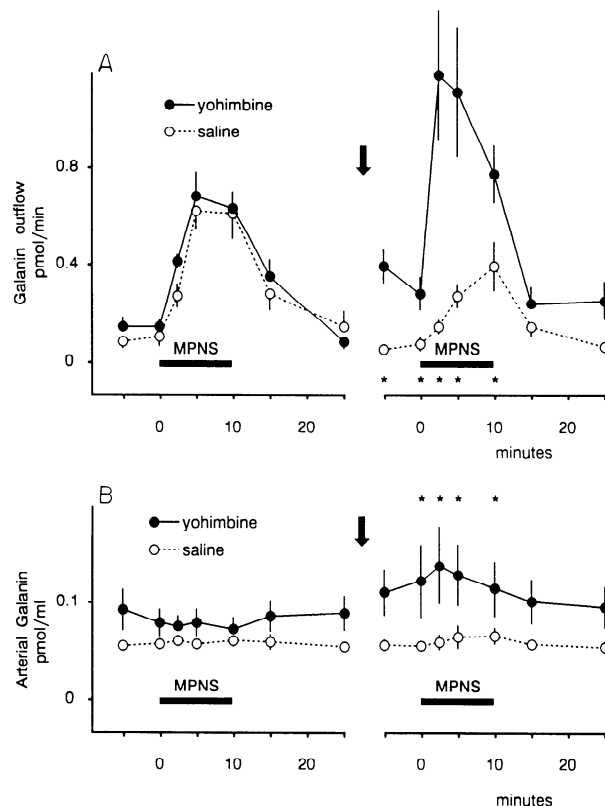

Fig. 3. Effect of intravenous administration of the α_2-selective adrenoceptor antagonist yohimbine (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$) on pancreatic outflow of galanin-like immunoreactivity (*A*, ●, n = 6) and arterial galanin levels (*B*, ●, n = 6) before, during, and after pancreatic nerve stimulation (MPNS). ○, Data from control experiment. Data are averages ± SE. Nerve stimulation is indicated by horizontal bar at the bottom of each graph. Yohimbine was infused for 10 min starting 15 min before the second nerve stimulation. * $P < 0.05$, significant change from values at comparable time points in control experiment.

Experiment 3: effects of α_2-adrenergic agonism. The aim of the third experiment was to determine whether inhibition of pancreatic NE outflow via activation of α_2-adrenoceptors was accompanied by decreased outflow of pancreatic galanin. The α_2-selective adrenoceptor agonist clonidine (20 $\mu g/kg$) was intravenously administered by a single injection after two consecutive nerve stimulations, 15 min before a third nerve stimulation. The control experiment consisted of the three consecutive nerve stimulations of *experiment 1*. This experimental design was chosen to minimize the number of animals needed for the total study. The results are depicted in Table 1 and in Figs. 5 and 6. Administration of clonidine led to a slight but nonsignificant reduction in stimulated pancreatic outflow of both GLIR and NE (maximal stimulated outflow reduced from 0.42 ± 0.18 to 0.20 ± 0.04 pmol/min for GLIR, and from 59.4 ± 30.3 to 27.2 ± 13.0 pmol/min for NE). Femoral artery levels of NE were markedly decreased after administration of clonidine (from 1.24 ± 0.34 to 0.10 ± 0.03 pmol/ml; the decrease was significant at all time points). In contrast, arterial GLIR levels remained unchanged.

Correlation between NE and galanin outflow. The data obtained in *experiments 2* and *3* were used to calculate the correlation between galanin and NE outflow, and their modulation by α_2-adrenoceptor mechanisms. For each
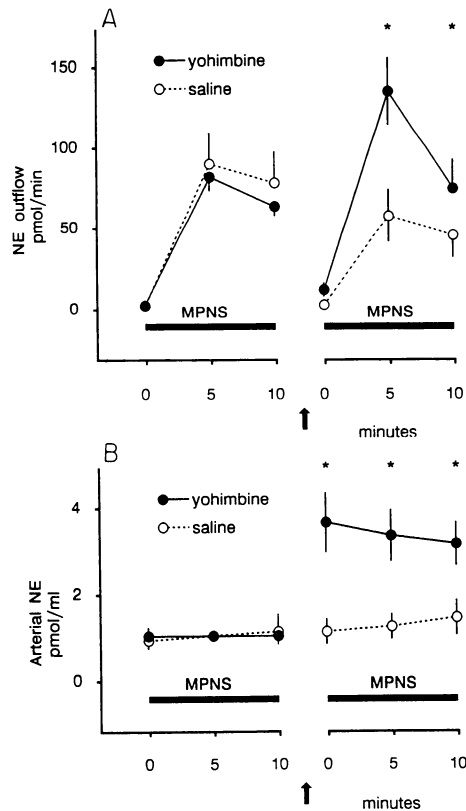
Fig. 4. Effect of intravenous administration of the α_2-selective adrenoceptor antagonist yohimbine (50 $\mu g \cdot kg^{-1} \cdot min^{-1}$) on pancreatic outflow of norepinephrine (NE) (A, ●, $n = 6$) and arterial NE levels (B, ●, $n = 6$) before and during pancreatic nerve stimulation (MPNS). Data are expressed as in Fig. 3.

Fig. 5. Effect of intravenous administration of the α_2-selective adrenoceptor agonist clonidine (20 $\mu g/kg$) on pancreatic outflow of galanin-like immunoreactivity (A, ●, $n = 4$) and arterial galanin levels (B, ●, $n = 4$) before, during, and after pancreatic nerve stimulation (MPNS). ○, data from control experiment. Data are averages ± SE. Nerve stimulation is indicated by horizontal bar at the bottom of each graph. Clonidine was intravenously injected after 2 consecutive nerve stimulations, 15 min before the third stimulation.

individual dog ($n = 10$) the average outflow was calculated from the samples taken at $t = 5$ and 10 min during nerve stimulation. Figure 7 shows the correlation between the absolute outflow of galanin and NE during nerve stimulation after administration of yohimbine or clonidine. In Fig. 8, each point represents the change in stimulated outflow of galanin and NE, induced by administration of yohimbine or clonidine, and is expressed as percent change in outflow before and after drug treatment. The correlations ($r = 0.84$ and $r = 0.86$, respectively) were highly significant ($P < 0.001$).

DISCUSSION

Stimulation of the sympathetic nerves to the pancreas inhibits insulin and increases glucagon release (3, 28, 31), responses that are presumably mediated by the sympathetic neurotransmitter norepinephrine. However, pancreatic arterial infusions of different doses of NE failed to reproduce the inhibition of basal insulin release seen during sympathetic pancreatic nerve stimulation in anesthetized dogs (3). Furthermore, combined α- and β-adrenoceptor blockade had little effect on these neurally induced changes of pancreatic islet hormone secretion (8). Because galanin-like immunoreactivity has been localized in pancreatic islet nerves (1, 7), and because galanin is a sympathetic neurotransmitter, released during stimulation of the thoracal splanchnic nerves (9), and because synthetic galanin potently inhibited insulin release (7, 19), galanin became a candidate mediator of the nonadr-
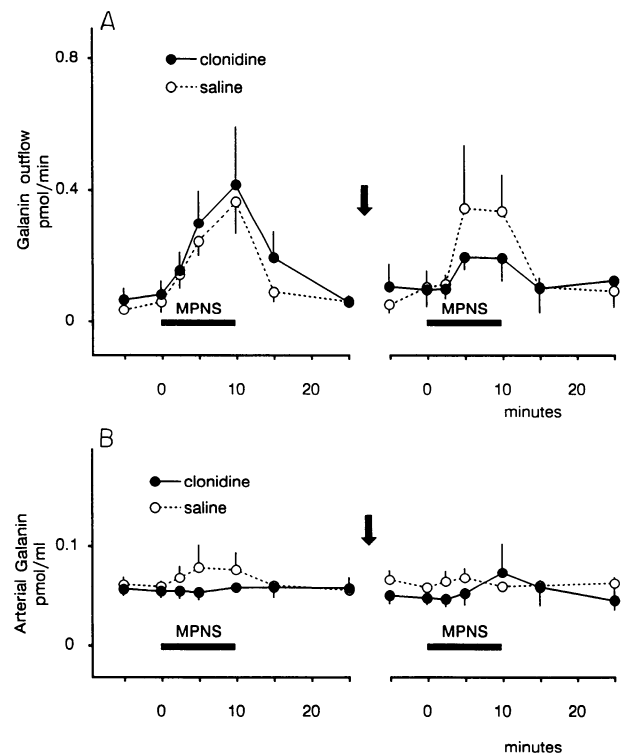
energic, sympathetic influences on the islets (10). On that basis, we hypothesized that galanin is a functional sympathetic neurotransmitter in the endocrine pancreas, co-released with NE from noradrenergic nerve endings (10). Our recent findings that stimulation of the mixed pancreatic nerve leads to the release of quantities of galanin sufficient to influence islet function (11) is compatible with this hypothesis.

In the present experiments, electrical stimulation of the mixed pancreatic nerves elicited an increase of both NE and galanin spillover into the superior pancreatic duodenal vein, and the pancreatic release of galanin paralleled that of NE in several circumstances. Furthermore, consecutive nerve stimulation elicited similar temporal secretory patterns of NE and galanin. NE and galanin spillover were significantly lower during the second and third stimulation compared with the first. These similar changes show that electrical nerve stimulation equally affected the release of NE and galanin and seem to confirm the hypothesized co-release of NE and galanin from the sympathetic nerves innervating the endocrine pancreas.

The main goal of the present study was to investigate whether the outflow of pancreatic galanin during sympathetic nerve stimulation is subject to the same presynaptic adrenergic regulatory mechanisms that have been established for NE. This would strongly suggest that NE and galanin are co-released. Precedence for such presynaptic adrenergic regulation of sympathetic cotransmitters
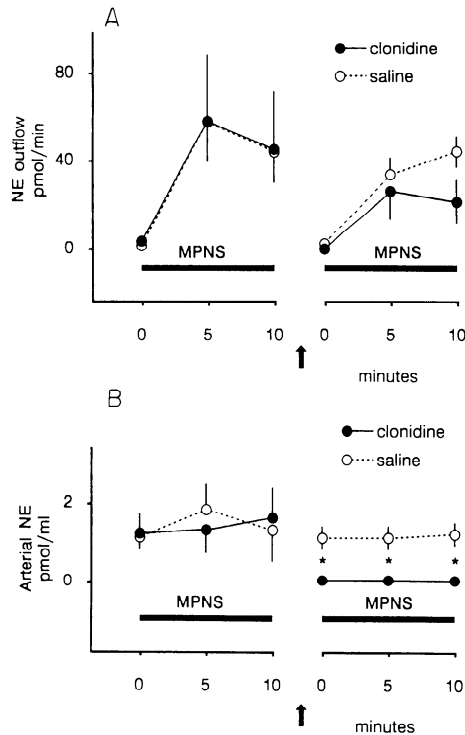
Fig. 6. Effect of intravenous administration of the α_2-selective adrenoceptor agonist clonidine (20 μg/kg) on pancreatic outflow of norepinephrine (NE) (A, ●, n = 4) and arterial NE levels (B, ●, n = 4) before and during pancreatic nerve stimulation (MPNS). Data are expressed as in Fig. 5. * P > 0.05, significant change from values at comparable time points in control experiment.
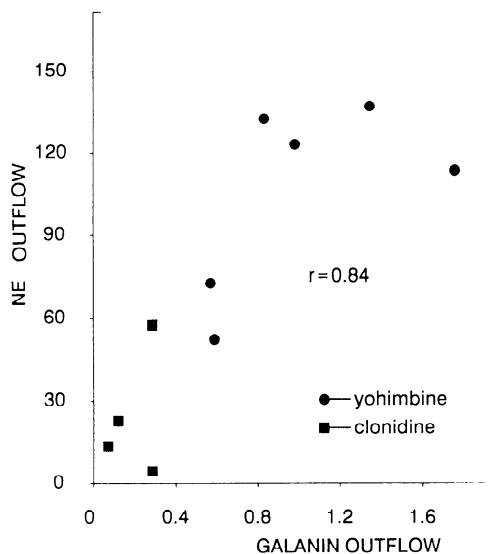

Fig. 7. Correlation between stimulated outflow of galanin-like immunoreactivity and norepinephrine (NE) after administration of yohimbine or clonidine. Each point represents for an individual dog (n = 10) the average outflow from the samples taken at t = 5 and 10 min during nerve stimulation.
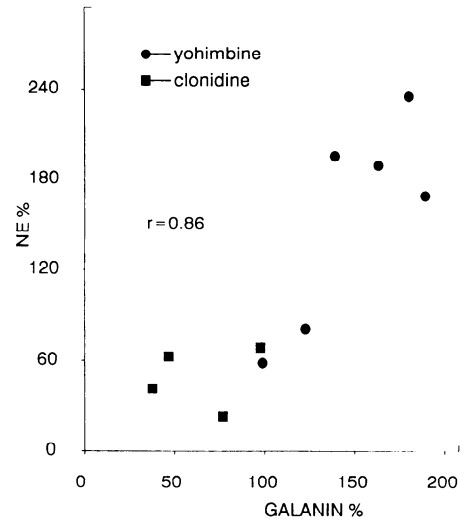

Fig. 8. Correlation between percent change in stimulated outflow of galanin and norepinephrine (NE) before and after administration of yohimbine or clonidine and expressed as percent change in outflow before and after drug treatment. Each point represents for an individual dog (n = 10) the relative change in stimulated outflow averaged from the samples taken at t = 5 and 10 min during nerve stimulation.

was already obtained for neuropeptide Y, co-released with NE from sympathetic nerves innervating cardiovascular endothelial cells (20, 21, 30). In experiment 2, administration of the α_2-selective adrenoceptor antagonist yohimbine significantly increased the baseline and stimulated spillover of both NE and galanin. Pharmacological and physiological studies (17, 22, 26, 29) provided evidence that the changes in NE spillover are mainly due to

blockade of inhibitory α_2-adrenoceptors on the presynaptic nerve endings of the peripheral sympathetic nerve system rather than to a central action of yohimbine (25). In addition, it is unlikely that any effect of yohimbine to change centrally directed sympathetic outflow would have much influence on neurotransmitter release induced by direct electrical stimulation. The strikingly identical alterations in NE and galanin spillover after α_2-adrenoceptor blockade therefore suggest that the release of galanin during activation of the sympathetic nervous system is equally modulated by presynaptic regulatory mechanisms, which may be considered a strong argument for co-release of galanin with NE from the sympathetic nerve endings.

The concentrations of NE and GLIR in the femoral artery did not change after electrical stimulation of the pancreatic nerve. This is in agreement with previous findings (9) and indicates that pancreatic outflow does not significantly contribute to the concentrations of sympathetic neurotransmitters in the general circulation. However, blockade of α_2-adrenoceptors markedly increased arterial plasma levels of NE and GLIR (experiment 2), both before and during nerve stimulation. With regard to NE, these results suggest an enhanced spillover of NE from other, nonpancreatic sympathetic nerve endings in the body. In particular, areas that make a major contribution to the systemic NE levels, such as the endothelial cells in the vasculature, must have potentiated their NE release in response to the systemic administration of yohimbine (12–14). The increased arterial GLIR concentrations point to extrapancreatic sources of galanin, influenced by α_2-adrenoceptor blockade. This finding confirms a previous report (9) of increases of nonpancreatic galanin in arterial plasma. In that study (9), general stimulation of the peripheral sympathetic nervous system caused a significant increase in circulating galanin. It was suggested that the gastrointestinal tract, the liver, and/or the adrenals may have been primarily

responsible for the increase in arterial galanin levels (4, 9, 11). However, the physiological function of nonpancreatic galanin remains unclear.

Administration of clonidine, an α_2-selective adrenoceptor agonist, caused only a minor reduction of pancreatic spillover of NE and galanin during nerve stimulation, which is in accordance with the idea that sympathetically released endogenous NE may have already activated the inhibitory α_2-adrenoceptors on the presynaptic nerve endings that reduce neurotransmitter outflow (17, 27, 29). If so, clonidine would be capable of exerting only a small additional effect. On the other hand, clonidine dramatically decreased arterial levels of NE. Part of the decrease of arterial NE might be mediated by activation of presynaptic α_2-adrenoceptors, since they are unlikely to be saturated by the low synaptic NE concentrations accompanying maintenance of sympathetic tone. Additionally or alternatively, the decrease in plasma NE concentrations in the femoral artery in the present experiment could also be explained by a centrally mediated reduction in sympathetic activity, since clonidine is known to decrease sympathetic outflow via both central (16, 18) as well as peripheral (32) pathways. The central effect of clonidine is likely to be dominant, since the administration of clonidine also markedly suppressed epinephrine levels in the femoral artery in the present study (Table 2), and the adrenal chromaffin cells are not thought to possess α_2-adrenergic inhibitory receptors analogous to those on the sympathetic nerve endings.

The present experiments do not not exclude that galanin might be released from nonadrenergic sympathetic pancreatic nerves containing solely neuropeptides that are influenced by adrenergic presynaptic regulatory mechanisms identically to the noradrenergic nerves. The possibility that sympathetic activation and consequently NE outflow might have had a postsynaptical effect on an endogenous source of galanin in the endocrine pancreas leading to increase in galanin secretion seems less probable, since combined administration of α- and β-adrenoceptor antagonists could not prevent the reduction in insulin secretion caused by activation of the sympathetic nervous system (8).

In summary, the main findings of the present study were 1) stimulation of the mixed pancreatic nerve led to a simultaneous increase of both NE and galanin spillover into the pancreatic vein, and similar secretory patterns

occurred during consecutive stimulation; 2) pancreatic NE and galanin spillover were both markedly increased after administration of the α_2-selective adrenoceptor antagonist yohimbine and modestly decreased after α_2-adrenergic stimulation with clonidine; and 3) yohimbine produced parallel changes in the systemic levels of both NE and galanin. Together with available anatomical data (1, 7), these results suggest colocalization and co-release of galanin and NE from sympathetic pancreatic nerves and therefore support the hypothesis that galanin is a sympathetic cotransmitter in the canine endocrine pancreas.

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Table 2. *Plasma epinephrine concentrations in the femoral artery before (t = 0 min) and during (t = 5 and 10 min) pancreatic nerve stimulation with and without administration of 20 μg/kg clonidine (experiment 3)*

Time, min	Plasma Epinephrine, pmol/ml	
	Control	Clonidine injection
0	0.89±0.48	0.13±0.03*
5	1.03±0.52	0.16±0.02*
10	1.02±0.48	0.14±0.02*

Values are averages ± SE; n = 4 in both groups. * Significant ($P < 0.05$) decrease in plasma epinephrine levels compared with control group.

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