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Effect of Pancreatic Polypeptide Family on Cardiovascular Muscle Contractility: 1. Interactions with cyclic nucleotide activators and K^+ channel openers in canine cerebral arteries

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ABSTRACT

The objectives of the present experiments were to characterize the effects of the peptides belonging to the pancreatic polypeptide family on the contractility of cerebral arteries and to observe the interactions of these peptides with the cyclic nucleotide activators and the potassium channel openers.

Dogs of either sex, 20~30 Kg in weight, were sacrificed. Basilar and middle cerebral arteries from brain were isolated and prepared for myography in the PSS equilibrated with 95% O_2 and 5% CO_2 at 37℃. The endothelial cells of the spiral strips were removed by CHAPS solution (0.3% w/v, 15 seconds).

1. PP, PYY and NPY contracted the arterial strips concentration-dependently with a rank order of potency of PYY>NPY>PP. These peptides were 20 to 200 times more potent than norepinephrine, and only PYY showed a greater potency than 5-HT.

2. Cyclic nucleotide activators, forskolin (for cAMP) and sodium nitroprusside (for cGMP) reduced the basal tone and inhibited the PP-, PYY- and NPY- induced contractions by concentration-dependent manners. Forskolin was more potent in reducing basal tone than sodium nitroprusside.

3. Potassium channel openers, RP 49356, P 1060 and BRL 38227 reduced the basal tone concentration-dependently and tended to inhibit the PP-, PYY- and NPY- induced contractions. Notably, BRL 38227 with low concentration (0.1 μM) enhanced the contractions induced by those peptides while P 1060 inhibited the contractions concentration-dependently.

4. The combinations of the cyclic nucleotide activators and the potassium channel openers were slightly additive in reducing the basal tone. P 1060 and BRL 38227 enhanced the relaxant effect of sodium nitroprusside significantly. On the PYY-induced contration (0.1 μM), K^+ channel openers tended to inhibit the inhibitory actions of forskolin and sodium nitroprusside. P 1060 and BRL 38227 antagonized the inhibitory action of sodium nitroprusside significantly.

The results of the present study may be summarized that in canine cerebral arteries, not only NPY but also PYY may play a role in a cerebrovascular spasm, and intracellular concentration of either cAMP or cGMP may be involved in the mechanism of vasoconstrictive actions of these peptides, which may be affected either positively or negatively by a K^+ channel opener.

Key Words: Pancreatic polypeptide, Peptide YY, Neuropeptide Y, Forskolin, Sodium nitroprusside, RP 49356, P 1060, BRL 38227, cyclic AMP, cyclic GMP, Potassium channel

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INTRODUCTION

Pancreatic polypeptide family (PPF) is a group of homologous 36 amino acid peptides. They are pancreatic polypeptide (PP), peptide YY (PYY) and neuropeptide Y (NPY). These peptides serve as gastrointestinal hormones and neurotransmitters. PP is found within the pancreas (Langsolw *et al.*, 1973), and it regulates the gastric and pancreatic exocrine functions and gallbladder motility (McGuigan, 1989). NPY is present in both central and peripheral neurons, whereas PYY is mainly expressed in endocrine cells in the lower bowel. Both NPY and PYY are potent vasoconstrictors in various organs (Corder *et al.*, 1987; Corder & Withrington, 1988; Franco-Cereceda, 1989). However, these peptides regulate gastrointestinal function by effects on blood flow, by modulating neural control mechanism (Sheikh, 1991).

Some authors reported the existence of PYY-positive fibers in the lower brain stem (Lundberg *et al.*, 1984) and PYY-like immunoreactivity in brain tissue (Ekman *et al.*, 1986; Batten *et al.*, 1990), which suggested that PYY also may play a physiological role in central nervous system. Recently, the existences of NPY receptor and PYY receptor in the brains of rat (Leslie *et al.*, 1988; Martel *et al.*, 1990) and dog and/or pig (Oya *et al.*, 1989; Inui *et al.*, 1989; Shigeri *et al.*, 1991) were proved. The vasoconstrictive actions of NPY and PYY were also observed in feline (Edvinsson, 1985) and rabbit (Lee KY *et al.*, 1990) cerebral arteries, and Juul *et al.* (1990) suggested a possible NPY involvement in cerebral vasoconstriction after subarachnoid hemorrhage. PP is not produced in the brain, but a high concentration of PP receptors was found in a particular region of rat brain (Whitcomb *et al.*, 1990).

Many investigators (Edvinsson, 1985; Andriantsitohaina & Stoclet, 1988; Duesler *et al.*, 1990; Lee *et al.*, 1990) discussed the calcium dependent vasoconstrictor actions of NPY and PYY. At the same time, some authors suggested that a reduction of intracellular concentrations of cyclic AMP (cAMP) or cyclic GMP (cGMP) may be included in the mechanisms of actions of PYY (Wiley *et al.*, 1991) and NPY (Motulsky & Michel, 1988; Aakerlund *et al.*, 1990; Lobaugh & Blackshear, 1990).

Recently, a new class of smooth muscle relaxant, potassium channel openers has been introduced. Opening of K^+ channels appeared to underlie their relaxations of a variety of smooth muscles (Cook 1988). K^+ channel openers are powerful smooth muscle relaxants with *in vivo* hypoten-sive and bronchodilator activity (Edwards & Weston, 1990). Stockbridge *et al.* (1991) suggested that such drugs, acting on cerebral arterial smooth muscle cell potassium channels, may be of some benefit in the treatment of cerebral vasos-pasm following subarachnoid hemorrhage.

Based on those reports, we were interested in the possibility of an involvement of PYY as well as NPY in the causative agents of cerebral vasospasm and further cerebrovascular accidents, and we observed the effects of PPF on the contractility of dog cerebral vessels. The interactions of the PPF with the cyclic nulceotide activators, forskolin (for cAMP) and sodium nitroprusside (for cGMP) were observed to determine the dependences of the actions of PPF on the cyclic nucleotide. The interactions of PPF with the K^+ channel openers, RP 49356, P 1060 and BRL 38227 were also investigated to see if/how they could inhibit the PPF-induced contractions.

MATERIALS AND METHODS

Preparation of arterial strips

Dogs of either sex, 20~30 Kg in weight, were sacrificed by a blow on occipital area and exsanguination followed by immediate decapitation. The scull was rapidly opened, and the brain was removed and soaked in ice-cold Krebs-Henseleit's physiological salt solution (PSS). Basilar and middle cerebral arteries were isolated and immersed in the PSS equilibrated with 95% O_2 and 5% CO_2 at about 4℃. The composition of the medium was (mM): Nacl 120, $CaCl_2$ 2.5, KCl 4.6, $NaHCO_3$ 23.8, KH_2PO_4 1.17, $MgSO_4$ 1.2 and Dextrose 10. The arteries were cleaned of connective tissue and side branches and cut into spiral strips of 1~1.5 mm wide and 12~15 mm long. The endothelial cells of the arterial strips were removed by

CHAPS (3-[(3-cholamidopropyl)-dimethylamino]-1-propanesulfonate) solution. CHAPS (0.3% w/v) had been previously reported to destroy the endothelium from the isolated mesenteric arterial bed (Douglas & Hiley, 1990) and the renal arteries of rat *in vivo* (Oyekan *et al.*, 1991). The vascular muscle strips were immersed in 0.3% CHAPS solution for 15 seconds and were washed with PSS. Destruction of the endothlium was confirmed by addition of acetylcholine (10 μM) into the baths; muscle strips were not relaxed by acetylcholine. Arteries were tied with silk suture string on both ends, and mounted in water-jacketed muscle baths of 1 ml working volume maintained at 37℃ and bubbled with 95% O_2 and 5% CO_2 (pH 7.4) Before the arterial strips were mounted, inner surface of the muscle bath was carefully rinsed with 2% bovine serum albumin solution to avoid binding of peptides to the plastic surface.

Tension recording of arterial strips

At the beginning of each experiment, the strips were stretched to an initial tension of 1 g, perfused with slow dribbling of PSS for 1hour, and allowed to equilibrate for at least half an hour. Muscle tensions were measured isometrically using force-displacement strain gages (model FT-03, Grass Instument Co.) and recorded on a polygraph (model 79E, Grass Instrument Co.)

The concentration-responses to PP, PYY and NPY were observed by cumulative additions of each peptide. Cumulative concentration-response of norepinephrine (NE) and 5-hydroxytryptamine (5-HT) was observed as a reference to compare. Responses of the arterial stips to potassium channel openers and cyclic nucleotide activators were observed by cumulative additions of the relaxant drugs. Washing by continuous slow dribbling and equilibration procedure were repeated for the strips which were contracted by the peptides. When the arterial strips equilibrated, one of the relaxant drugs of P 1060 (0.1, 1 and 10 μM), RP 49356 (0.1, 1 and 10 μM), BRL 38227 (0.1, 1 and 10 μM), forskolin (10, 30, and 300 nM), and sodium nitroprusside (10, 30, and 300 nM) was added into a bath. Then cumulative administration of PP, PYY or NPY was repeated in the presence of those muscle relaxants. In the experiments of the concentration-response to peptides in the presence of various inhibitors, the sequence of control and experiments was randomly switched to eliminate the possibility of any influence of the previous exposure to any peptide or relaxant drug. Interactions of potassium channel openers and cyclic nucleotide activators on the basal tone and PYY- induced contraction were observed. PYY (0.1 μM) was administered in the presence and absence of combinations of a potassium channel opener and a cyclic nucleotide activator. At the end of each series of experiments which include the relaxation response, EDTA (ethylenediamine tetraacetic acid) in a concentration of 30 mM was applied to preduce the maximum relaxation.

Drugs and solutions

Lyophilized preparations of pancreatic polypeptide (Sigma), peptide YY (Sigma) and neuropeptide Y (Sigma) were reconstituted by additions of demineralized water, and the stock aliquots for 10 μM (the final concentration to obtain when 20 μl was administered in 1 ml bath) were stored in a freezer at −20℃. The frozen aliquots were melted and diluted with demineralized water for every experiment. Stock solutions of P 1060 (Leo), RP 49356 (Rhone-poulenc Rorer), BRL 38227 (Smithkline Beecham) for the final concentration of 10^{-4} M in 40~60% ethanol/water were stored in 4℃, and then diluted with distilled water for daily use. Norepinephrine (Sigma), 5-HT (Sigma), forskolin (Sigma), sodium nitroprusside (Sigma), CHAPS (Sigma) and EDTA (Sigma) were diluted with demineralized water. Control experiments established that effects were not the result of vehicles.

Data analysis

Concentration-response results have been expressed as a percentage of the maximum possible contraction or the maximum relaxation. To estimate the maximal effect and EC_{50}, the concentration-response curves of peptides and 5-HT were analyzed by fitting the logistic equation:

$$E=(Emax \cdot [C])/(K_D+[C]),$$

where E is the increase in tension, C is the final concentration of the agonists in the bath, and K_D is the dissociation constant whose value reflects

the concentration for a half-maximal response. The effects of the relaxant durgs were expressed as a percentage of the relaxation caused by 30 mM EDTA. pD_2 was calculated by the equation, $pD_2 = -\log(EC_{50})$. Some results were expressed as mean±standard error of the mean, and comparison between means was carried out by Student's t-Test. A probability of <0.05 was considered significant.

RESULTS

Contractile response to peptides of the PP family

The contractile responses to PP, PYY and NPY in both basilar and middle cerebral arteries were concentration-dependent, reproducible and consistent in patterns (Fig. 1). There was no differ-

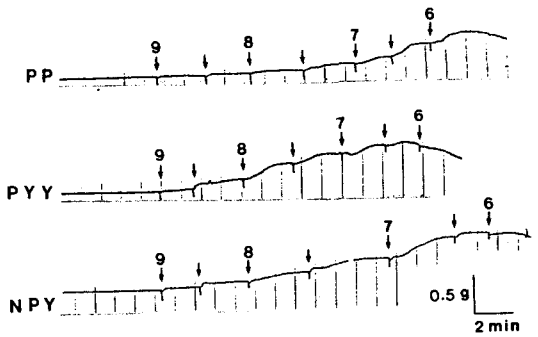

Fig. 1. Typical tracings of the cumulative concentration-responses of canine cerebral arterial strips to PP, PYY and NPY. Numbers above arrows mean -log[peptide], and the arrows without number between every dose point indicate half-log concentration points.

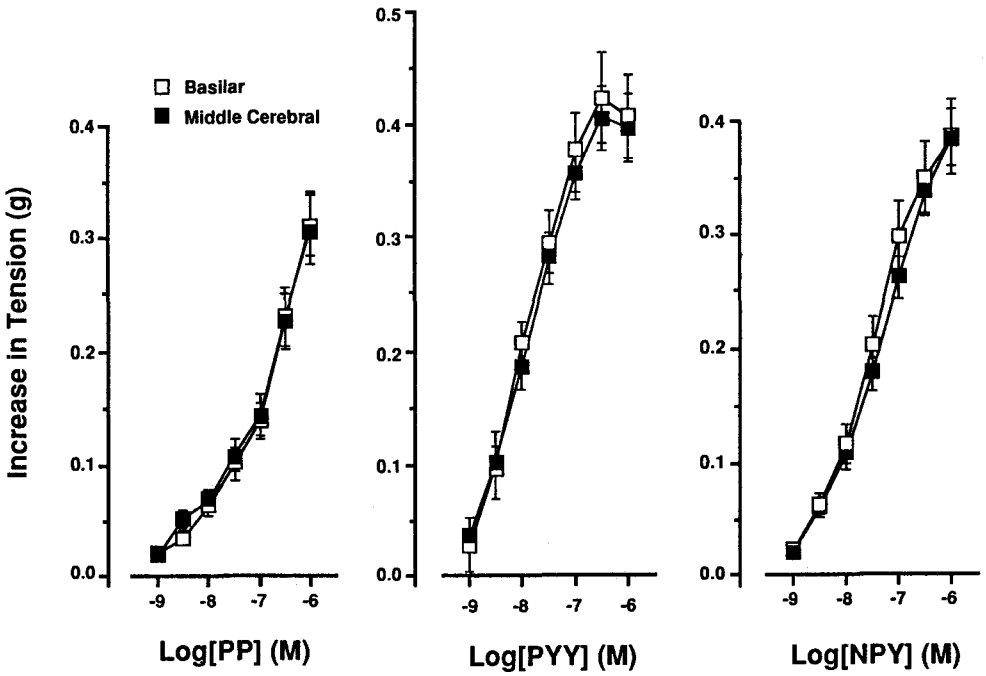

Fig. 2. Concentration-dependent contractions of isolated canine basilar and middle cerebral arteries by PP, PYY and NPY. Values are expressed as Mean±SE of the increment of tension(g). There were no significant difference between the responses of basilar and middle cerebral arteries. The EC_{50} values of PP, PYY and NPY were approximately o.1 μM, 0.01 μM and 0.03 μM, respectvely(n=26 for each group, see Table 1).

Table 1. Summary of concentration-response data obtained with norepinephrine, 5-HT and pancreatic polypeptide family on the cerebral arterial strips isolated from dog

Drug	Artery	Emax(g)	$EC_{50}(\mu M)$	pD_2
NE(n=6)	Middle cerebral	0.33±0.03	2.0±1.6	5.7
5-HT(n=7)	Middle cerebral	0.54±0.03	0.05±0.01	7.3
PP(n=26)	Basilar	0.33±0.03*	0.1±0.04	7.0
	Middle cerebral	0.31±0.04	0.07±0.03	7.1
PYY(n=26)	Basilar	0.42±0.01*	0.01±0.001*	8.0
	Middle cerebral	0.41±0.01	0.01±0.001	7.9
NPY(n=26)	Basilar	0.38±0.01*	0.03±0.003	7.6
	Middle cerebral	0.38±0.02	0.03±0.007	7.5

NE=norepinephrine, 5-HT=5-hydroxytryptamine, PP=pancreatic polypeptide, PYY=peptide YY, NPY =neuropeptide Y. Emax, the maximal increments of tension(ΔT) and EC_{50}, the concentration which produced half-maximal contraction were obtained by fitting of data to the logistic equation given in the text. pD_2 is $-\log EC_{50}$.

*$p<0.05$; significantly different from 5-HT.

ence between the responsivenesses of basilar and middle cerebral arteries to each peptide (Fig. 2). The maximum contraction elicited by PYY (n=26 for each arterial group) was 0.41±0.01 g on middle cerebral artery and 0.42±0.01 on basilar artery, by NPY (n=26 for each arterial group) was 0.38±0.02 g and 0.38±0.01 g and by PP (n=26 for each arterial group) was 0.31±0.04 g and 0.33 ±0.03 g. All these maximum contractilities were similar to that induced by NE (n=6) but lower than that by 5-HT (n=7, $p<0.05$, Table 1) on middle cerebral artery, The EC_{50} value for PYY which was the most potent one in the pancreatic polypeptide family was $1\pm0.1\times10^{-8}$ M whereas that of 5-HT was $5\pm1\times10^{-8}$ M on middle cerebral artery, and the former was significantly more potent ($p<0.05$). PYY was 200 times more potent than NE (EC_{50}; $2\pm1.6\times10^{-6}$M) on middle cerebral artery. For all the following experiments, the strips from basilar and middle cerebral arteies were radomly grouped.

Relaxation by cyclic nucleotide activators

Figure 3 shows that forskolin and sodium nitroprusside produced similar relaxant effects on canine cerebral arteries. Forskolin relaxed the arterial strips from basal tone in the resting equilibrated state with 92.2±5.7% of Emax, and the

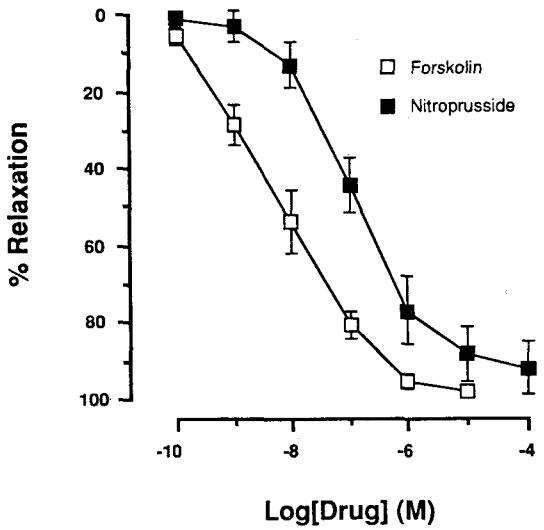

Fig. 3. Concentration-dependent relaxations of caine cerebral arterial strips by cyclic nucleotide activators, forskolin(for cAMP) and sodium nitroprusside(for cGMP). Values are expressed as Mean±SE of the % relaxation. The tension level of full relaxation by 30 mM EDTA was considered as 100% relaxation. The EC_{50} value of forskolin, 5±2.1 nM(n=7) were significantly($p<0.05$) lower than that of sodium nitroprusside, 98±15 nM(n=5).

Table 2. Summary of concentration-response data obtained with cyclic nucleotide activators and K^+ channel channel openers on the basal tone of the canine cerebral arterial strips

Drug	-Emax(%)	EC_{50}(μM)	pD_2
Forskolin(n=7)	92.2±5.7	0.005±0.002	8.3
Na nitroprusside(n=5)	88.8±2.1	0.98±0.15*	7.0
RP 49356(n=6)	89.2±6.3	4.4±1.0	5.35
P 1060(n=6)	84.6±7.7	1.6±0.6	5.8
BRL 38227(n=6)	80.3±5.7	1.0±0.3†	6.0

Emax, the maximal % change of basal tone (full relaxation by 30 mM EDTA was considered as -100%) and EC_{50}, the concentration which produced half-maximal relaxation were obtained by fitting of data to the logistic equation given in the text. pD_2 is $-\log EC_{50}$.

*$p<0.05$; significantly different from forskolin.

†$p<0.05$; significantly different from RP 49356.

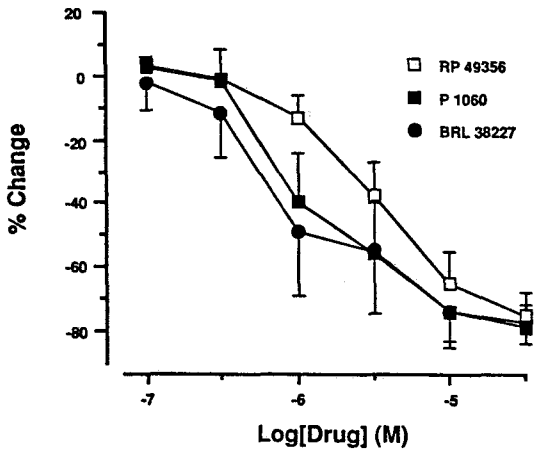

Fig. 4. Concentration-dependent relaxations of canine cerebral arterial strips by K^+ channel openers, RP 49356, P 1060 and BRL 38227. Values are expressed as Mean±SE of the % change of basal tone(n=6 for each group). The tension level of full relaxation by 30 mM EDTA was considered as -100% change. The EC_{50} values of RP 49356, P 1060 and BRL 38227 were 4.4±0.1 μM, 1.6±0.6 μM and 1±0.3 μM, respectively(see Table 2).

Emax for sodium nitroprusside was 88.8±2.1% (Table 2) where the maximum relaxation by 30 mM EDTA was considered as 100%. The EC_{50} values for forskolin and sodium nitroprusside were $5\pm2\times10^{-9}$ M and $9.8\pm1.5\times10^{-8}$ M, respectively, and those values were significantly different ($p<0.05$).

Relaxation by K^+ channel openers

Figure 4 shows that RP 49356, P 1060 and BRL 38227 produced similar relaxant effects in the cerebral arterial strips in a resting equilibrated state. The maximum relaxation by RP 49356 was 89.2±6.3%, it was at the similar level of 84.6±7.7% by P 1060 and 80.3±5.7% by BRL 38227. The rank order of potency was BRL 38227>P 1060>RP 49356. The EC_{50} values for BRL 38227 was $1\pm0.3\times10^{-6}$ M, for P 1060 was $1.6\pm0.6\times10^{-6}$ M and for RP 49356 was $4.4\pm1.0\times10^{-6}$ M.

Effects of cyclic nucleotide activators on the PPF-induced contractions

Forskolin suppressed the concentration-response curves of PP, PYY and NPY, concentration-dependently (Fig. 5). In the range of the concentration in this experiment, forskolin reduced the PP-indcued contrations significantly ($p<0.05$). The control value of 93% by PP (1 μM), which could not reach the level of the logistic Emax by curve fitting, was significantly reduced to 73% ($p<0.05$), 40% ($p<0.05$) and 8% ($p<0.05$) by 10 nM, 30 nM and 300 nM of forskolin, respectively. On both PYY- and NPY-induced contractions, forskolin reduced the Emaxs significantly. The Emax value of 96% for PYY in control group was reduced to 83% (nonsignificant) and 39% ($p<0.05$) by 30 nM and 300 nM of forskolin, respectively. The Emax value of 99% for NPY in control group was reduced to 64% ($p<0.05$) and 41% ($p<0.05$) by

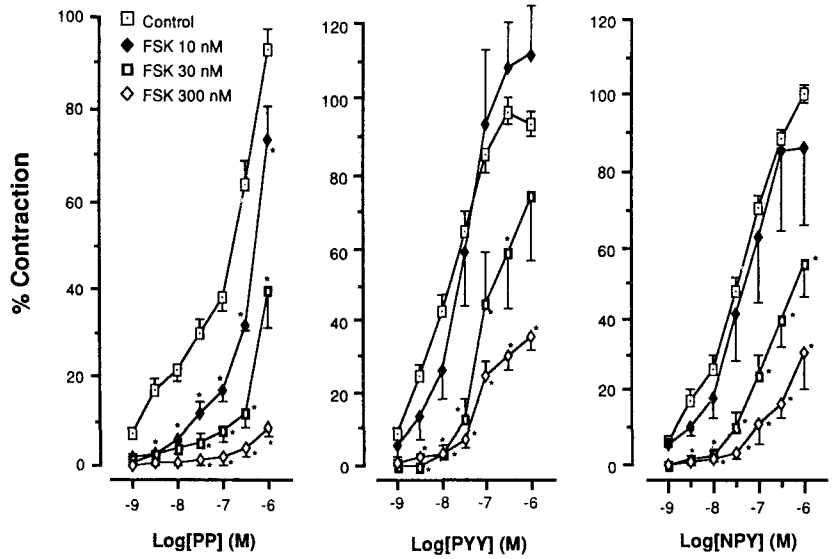

Fig. 5. Inhibitory effect of forskolin on PP-, PYY- and NPY- induced contractions of canine cerebral arterial strips. FSK abbreviates forskolin. Values are expressed as Mean±SE of the percentrage of the maximal value of control state obtained by fitting to the logistic equation given in the text.
*$p<0.05$; significantly different from control ($n=13$ for controls, 4 or 5 for each exp.).

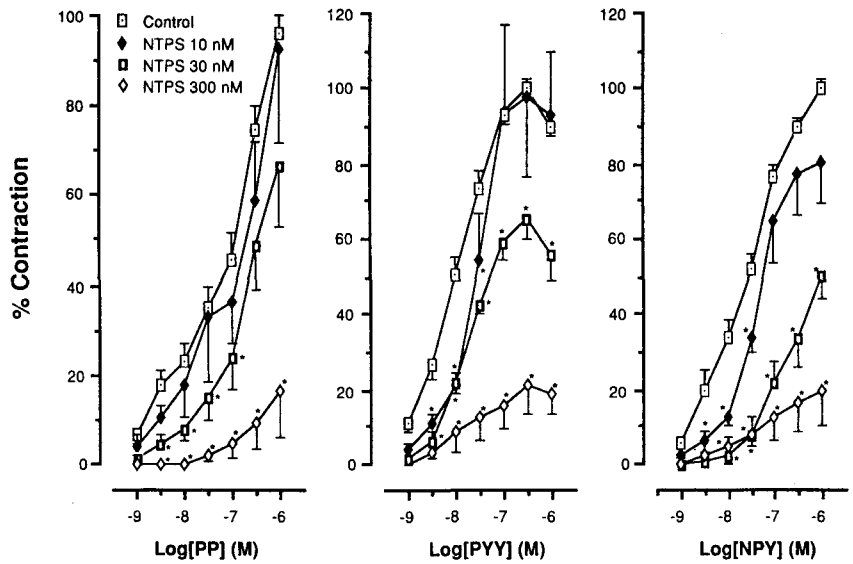

Fig. 6. Inhibitory effect of nitroprussdie on PP-, PYY- and NPY- induced contractions of canine cerebral arterial strips. NTPS abbreviates soidum nitroprusside. Values are expressed as Mean±SE of the percentage of the maximal value of control state obtained by fitting to the logistic equation given in the text.
*$p<0.05$; significantly different from control ($n=13$ for controls, 4 or 5 for each exp.).

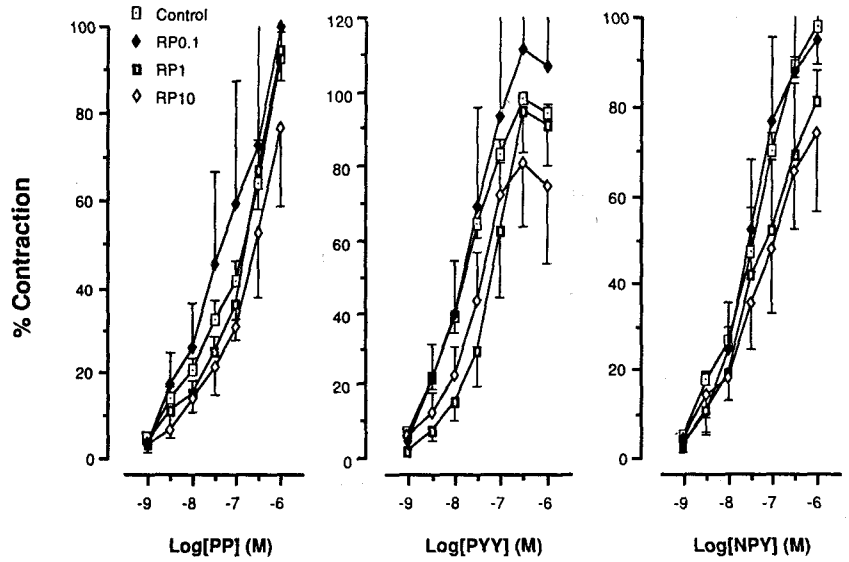

Fig. 7. RP 49356 showed a tendency of inhibition on the contractile actions of PP, PYY and NPY on the canine cerebral arterial strips, but the data did not reached to the level of statistical significance. RP 0.1, RP1 and RP10 mean the concentration of pretreated RP 49356; 0.1 μM, 1 μM and 10 μM, respectively. Values are expressed as Mean±SE (n=13~15 for controls, 4 or 5 for each exp.) of the percentage of the maximal value of control state obtained by fitting to the logistic equation given in the text.

30 nM and 300 nM of forskolin, respectively.

Sodium nitroprusside suppressed the concentration-response curve of PPF by almost the same feature as forskolin (Fig. 6). The Emaxs of 96% for PP was reduced to 25% ($p<0.05$), 98% for PYY was suppressed to 20% ($p<0.05$), and 98% for NPY was reduced to 19% ($p<0.05$), by sodium nitroprusside 300 nM. There was no evidence of difference in EC_{50} values in either experiment with forskolin or sodium nitroprusside.

Effects of K^+ channel openers on the PPF-induced contractions

As shown on figure 7, RP 49356 tended to reduce the PPF-induced contractions of the canine cerebral arterial stips, but the values could not reached to the level of statistical significance while the same concentrations of P 1060 with the same number of cases showed a significant suppression on the contractile responses to PPF (Fig. 8). High concentration (1 and 10 μM) of P 1060 reduced the Emaxs of PP (95%) to 58% and 51%, PYY (97%) to 78% and 50%, and NPY (96%)to 70% and 41%. Figure 9 shows the effect of BRL 38227 on the PPF-induced contractions. With a low concentration of 0.1 μM, BRL 38227 increased the Emax value of 95% for PP to 158% ($p<0.05$), 98% for PYY to 172% ($p<0.05$), and 98% for NPY to 127% ($p<0.05$). The higher concentations of 1 and 10 μM showed a slight tendency to reduce the contractile activity nonsignificantly.

Interactions by combination of a cyclic nucleotide activator and a K^+ channel opener

At a resting equilibrated state, an addition of RP 49356, P 1060 or BRL 38227 (3 μM each) evoked a reduction of basal tone (Fig. 11). A superimposed addition of forskolin or sodium nitroprusside (0.3 μM each) induced a further relaxation. A typical tracing from such an experiment was demonstrated on figure 10. On basal tone, the effects of the combinations of a K^+ channel opener and forskolin were slightly additive, but could not exceed the effect by forskoin alone (statisti-

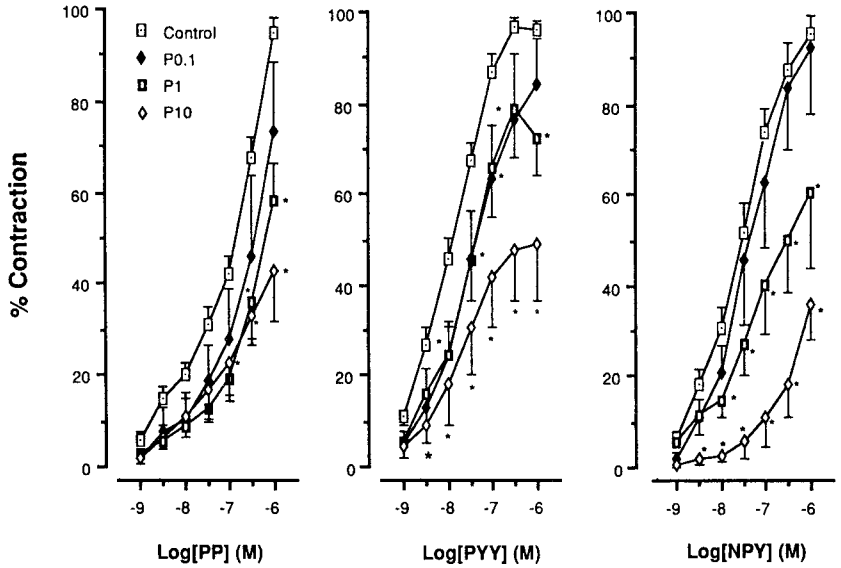

Fig. 8. Concentration-dependent inhibitions of P 1060 on PP-, PYY- and NPY- induced contractions of canine cerebral arterial strips. P0.1, P1 and P10 mean the concentration of pretreated P 1060; 0.1 μM, 1 μM and 10 μM, respectively. Values are expressed as Mean±SE of the percentage of the maximal value of control state obtained by fitting to the logistic equation given in the text.
*$p<0.05$; significantly different from control($n=15\sim17$ for controls, $4\sim6$ for each exp.).

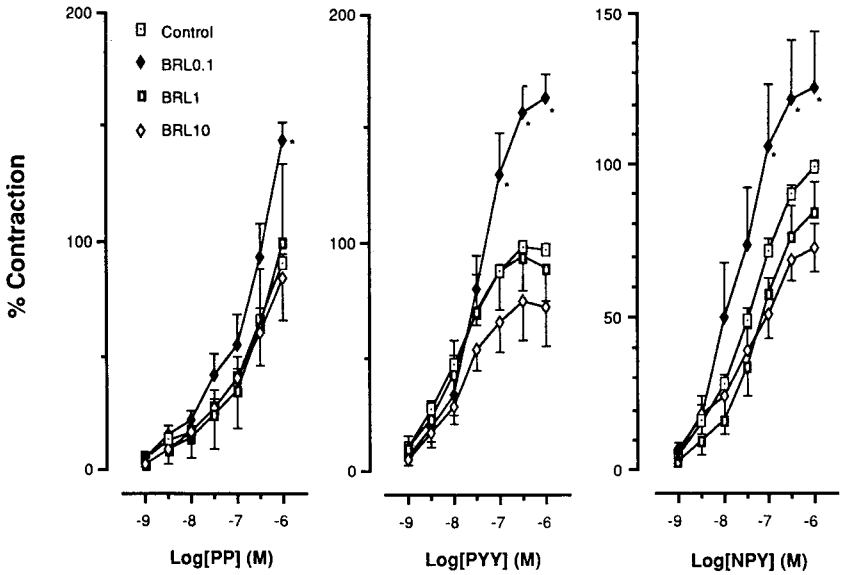

Fig. 9. Effect of BRL 38227 on PP-, PYY- and NPY- induced contractions of canine cerebral arterial strips. BRL0.1, BRL1 and BRL10 mean the concentration of pretreated BRL 38227; 0.1 μM, 1 μM and 10 μM, respectively. Note the significant enhancement of the concentration-responses to the peptides with low concentration (0.1 μM) of BRL 38227. Values are expressed as Mean±SE of the percentage of the maximal value of control state obtained by fitting to the logistic equation given in the text.
*$p<0.05$; significantly different from control ($n=14\sim17$ for controls, $4\sim7$ for each exp.).

cally nonsignificant). Pre-existing P 1060 and BRL 38227 significantly enhanced the relaxant effect of sodium nitroprusside from -60% to -87% ($p < 0.05$) and to -96%, respectively. RP 49356 was not appeared additive with either forskoin or sodium nitroprusside.

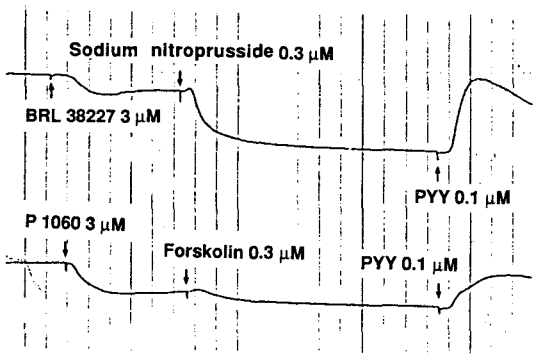

Fig. 10. Typical tracings of combined effects of a K^+ channel opener and a cyclic nucleotide activator

Figure 12 shows the effects of combinations of a K^+ channel opener and a cyclic nucleotide activator on the PYY-induced contraction. The K^+ channel openers inhibited the contraction by PYY 0.1 μM to the level of 54 to 76%. Forskolin and sodium nitroprusside were highly inhibitory to the PYY-induced contration allowing the muscle strips to contract only 25% and 16%. The pre-existing K^+ channel openers tended to antagonize the relaxant effects of forskolin and sodium nitroprusside. P 1060 prevented the inhibitory effect of sodium nitroprusside from 16% to 51% ($p<0.05$), and BRL 38227 inhibited the effect of sodium nitroprusside from 25% to 44% ($p<0.05$).

DISCUSSION

It is a classical concept that NPY is present in

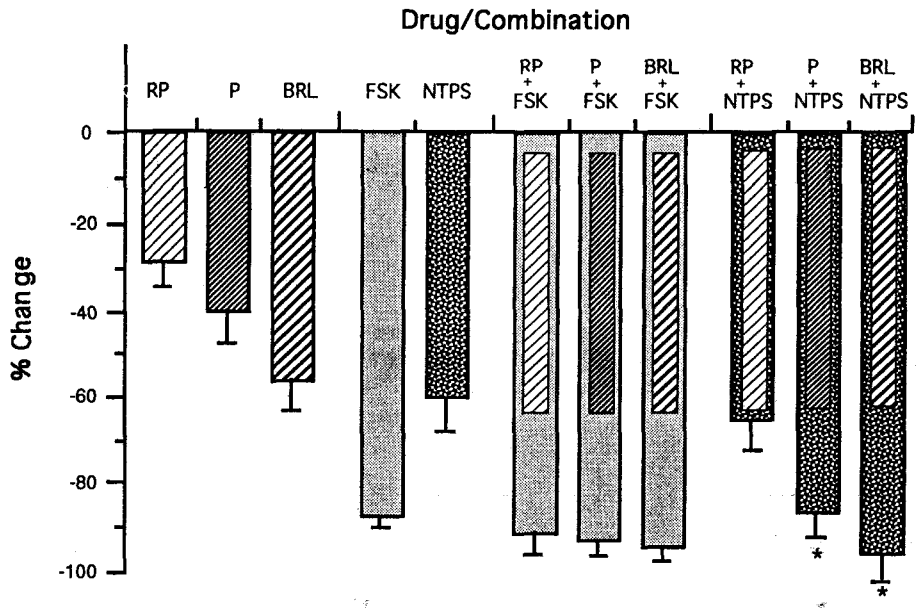

Fig. 11. Combined effects of the cyclic nucleotide inhibitors and K^+ channel openers on the basal tone of the canine cerebral arterial strips. RP=RP 49356, P=P 1060, BRL=BRL 38227, FSK=forskolin, NTPS=sodium nitroprusside. Values are expressed as Mean±SE of the % change of basal tone (n=6 for each group). The tension level of full relaxation by 30 mM EDTA was considered as -100% change.
*$p<0.05$; The pretreatment of P 1060 or BRL 38227 enhanced the nitroprusside-induced relaxation significantly.

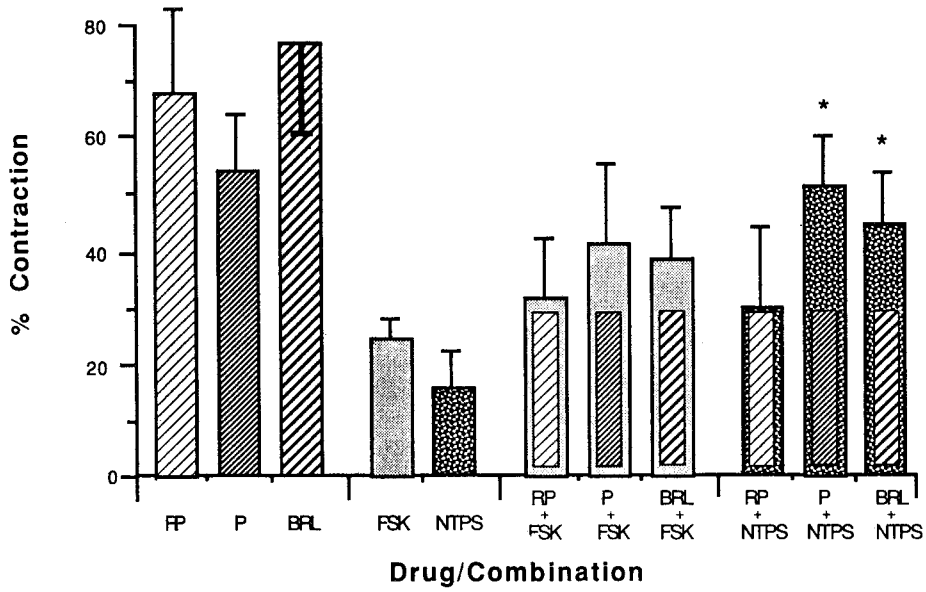

Fig. 12. Combined effects of the cyclic nucleotide inhibitors and K^+ channel openers on the PYY-induced contraction of the canine cerebral arterial strips. RP=RP 49356, P=P 1060, BRL=BRL 38227, FSK =forskolin, NTPS=sodium nitroprusside. Values are expressed as Mean±SE of the percentage of the contraction induced by PYY 0.1 μM at control state (n=6 for each group).
*$p<0.05$; The pretreatment of P 1060 or BRL 38227 prevented the nitroprusside-induced inhibition significantly.

both central and peripheral neurons, whereas PYY is mainly expressed in endocrine cells in the lower bowel (McGuigan, 1989). From some investigations in brain and cerebral vascular disease, the existence of PYY activity in central nervous system was reported. Broome *et al.* (1985) reported that there exists a small but widely distributed PYY system with cell bodies in the rostral medulla oblongata projecting to the lower brain stem and spinal cord. The concentration of PYY-like immunoreactivity in rat brain and spinal cord was determined by radioimmunoassay, and the highest concentrations were found in the cervical spinal cord and in the medulla oblongata (Ekman *et al.*, 1986). Furthermore, radiolabelled ligand binding studies demonstrated that specific receptors for PYY were present in the brain of rat (Leslie *et al.*, 1988; Walker & Miller, 1988), in the porcine and canine brains (Inui *et al.*, 1988), and in the brains of many animals (Okita *et al.*, 1991). The binding sites in the brain of such animals showed a high specificity for PYY and NPY, but not for PP or structurally unrelated peptides (Inui *et al.*, 1988).

It is well known that both NPY and PYY are potent vasoconstrictors in various organs (Corder *et al.*, 1987; Corder & Withrington, 1988; Franco-Cereceda 1989). Juul *et al.* (1990) indicated a possible NPY involvement in cerebral vasoconstriction as a result of their investigation on subarachnoid hemorrhage, and Wahlestedt *et al.*, (1992) emphasized that NPY and PYY often exert similar actions and binding profiles. In this study, not only NPY and PYY but also PP contracted the cerebral arterial strips. However. the PP receptor differs from neuropeptide Y and peptide YY receptors in its binding specificity and location, because PP is not produced in the brain, and the blood-brain barrier exculdes circulating peptides from most areas in the brain (Whitcomb *et al.*, 1990). So we may set aside the effect of PP for a further investigation. Unexpectedly, the contractile activity of PYY was more potent and more efficient than that of NPY. In comparison with the

effects of NE, a typical adrenergic neurotransmitter and 5-HT, a cerebral vasoconstrictor, PYY was 20 times more potent than 5-HT and 200 times more potent than NE. Tanaka and Chiba (1988) suggested that intraluminal 5-HT may be one of the possible etiological factors behind the chronic phase of vascular spasm following subarachnoid hemorrhage. Although a further quantitative measurement study should be needed to prove this hypothesis, PYY as well as NPY or 5-HT may be involved in cerebral vasoconstriction.

In various smooth muscle, cAMP and cGMP are an important mediator of relaxation responses (Nishimura & van Breemen, 1989; Chin & Hoffman, 1991; Word *et al.*, 1991; Lincoln & Cornwell, 1991). In rat anococcygeus muscle, forskolin caused relaxation and a sensitive increase in cAMP, and sodium nitroprusside produced relaxation and a rise in cGMP but not cAMP (Mirzazade *et al.*, 1991). Motulsky & Michel (1988) reported that NPY inhibited cAMP accumulation in human erythroleukemia (HEL) cells inhibiting adenylate cyclase. Many other investigators observed that NPY inhibited forskolin-induced activation of adenylate cyclase (Lundberg *et al.*, 1988; Aakerlund *et al.*, 1990; Schlicker *et al.*, 1991). NPY produced a rightward shift in the concentrationresponse curves for forskolin in guinea pig uterine artery (Morris, 1991). In the present study, forskolin inhibited the contractile effect of NPY, and this result is in agreement with the observation of Chernaeva (1990) in guinea pig vas deferens. It was presumed that in dog cerebral artery, the mechanisms of action of NPY included the inhibition of cAMP accumulation as it showed in HEL cells, and the activation of adenylate cyclase by forskolin antagonized the contraction. Forskolin inhibited the PYY- and PP- induced contractions also by almost the same pattern as the inhibition on NPY-induced contraction. Wiley *et al.* (1991) suggested that PYY can inhibit cAMP dependent release of acetylcholine in the isolated smooth muscle of guinea pig stomach, and we guess PYY exerts a similar contractile action like that of NPY. This presumption is supported by the suggestion that a common coupling mechanism of NPY/PYY receptors, i.e. to reduction of cAMP existed in cultured human neuroblastoma cells (Wahlestedt *et al.*, 1992). Although we could not find any report that described about the interrelationship between cGMP and mechanism of action of PPF, we observed that sodium nitroprusside, a guanylate cyclase activator inhibited PPF-induced contractions concentration-dependently. Clinically, sodium nitroprusside is a widely employed vasodilator activating guanlyate cyclase. Another vasodilator nicorandil also activates guanylate cyclase (Greenberg *et al.*, 1991; Kukovetz *et al.*, 1991), and activates K^+ channel (Edwards & Weston, 1990; Kukovetz *et al.*, 1991). Meisheri *et al.* (1991) suggested that a combination of these characteristics of the actions of nicorandil may contribute to the differences in the acute versus chronic hemodynamic profile. In a study of cerebral vasospasm after experimental subarachnoid hemorrhage in the dog, Harder *et al.* (1987) suggested that arteries exposed to subarachnoid blood are depolarized beacuse of a reduced resting K^+ conductance. On the other hand, a membrane hyperpolarization by a small elevation in external K^+ appeared to be an important mechanism for dilation of small cerebral arteries (McCarron *et al.*,1991). Since PYY and/or NPY is presumed to play an important role in the cerebral vasoconstriction, a consideration about the relationship between the vasoconstrictive action of PPF and K^+ channel should be helpful.

We investigated the effects of the hyperpolarizing vasodilators, RP 49356, P 1060 and BRL 38227 on the PPF-induced contractions. RP 49356, p1060 and BRL 38227 are tetrahydrothiopyrane, cyanoguanidine and benzopyran derivatives, respectively. All three K^+ channel activators employed in the present study tended to prevent the PPF-induced contrations of the arterial strips. RP 49356 could not significantly inhibit the PP-, PYY- or NPY- induced contractions. P 1060 distinctly inhibited PP-, PYY- and NPY- induced contractions concentration-dependently. A low concentration (0.1 μM) of BRL 38227 did not inhibit but rather enhanced PP-, PYY- and NPY-induced contraction up to around 150% of maximal contractions of controls. Higher concentrations (1 μM and 10 μM) of BRL 38227 tended to inhibit PPF-induced contractions. There are two different types of K^+ channels in smooth muscle, ATP-sensitive K^+ channel and Ca^{++}-activated K^+ channel. RP 49356 (Escande *et al.*, 1989) and BRL

38227 (Black *et al.*, 1990) activates the ATP-senstive K^+ channel, and P 1060 (Hu *et al.*, 1990) activates Ca^{++}-activated K^+ channel. In fact, we noticed that the concentration-response curves to PPF in the presence of low concentration of RP 49356 ran slightly above the control, but it was not significant statistically. Here, we have a question: Do the members of PPF exert different cellular responses interacting two different types of K^+ channels in dog cerebral arteries? McCarron *et al.* (1991) presumed that there may be differences in the types of K^+ channels that are activated by dilating mechanisms in small cerebral arteries, and Bryant *et al.* (1991) suggested that neuropeptide Y reduced the background rectifier K^+ current in guinea pig myocytes. To answer the question, we needed to observe the combined effects of a cAMP- or cGMP- activator and an ATP-sensitive K^+ channel- or Ca^{++}-activated K^+ channel- opener. For an experiment to observe the interaction of PPF with cyclic nucleotide activators and K^+ channel openers, we used PYY which was the most potent and the most efficient of three peptides.

The decrease in basal tone induced by K^+ channel openers was not showed a further decrease by superimposed addition of forskolin, but the relaxant effects of P 1060 and BRL 38227 was enhanced by sodium nitroprusside. The inhibitory effects of sodium nitroprusside on the PYY-induced contraction were antagonized by preexisting K^+ channel openers, especially P 1060 and BRL 38227. In an other word, K^+ channel openers and cyclic nucleotide activators acted on the basal tone synergistically, but antagonistically on the PYY-induced contraction. The data from this study are too sophisticated to make a brief conclusion, but anyway, both cAMP and cGMP even with K^+ channels may be related to the mechanism of contractile action of PPF on the cerebral arteries.

The results of the present study may be summarized that in canine cerebral arteries, not only NPY but also PYY may play a role in a cerebrovascular spasm, and intracellular concentration of either cAMP or cGMP may be involved in the mechanism of vasoconstrictive actions of these peptides, which may be affected either positively or negatively by a K^+ channel opener.

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= 국문초록 =

Pancreatic Polypeptide Family의 심혈관계 근육 수축성에 대한 약리학적 작용: I. 개의 뇌혈관에서 cyclic nucleotide활성제와 칼륨통로개방제와의 상호작용

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Pancreatic polypeptide family펩타이드들의 뇌혈관 평활근 수축성에 미치는 효과를 관찰하고, cyclic nucleotide 활성제 및 칼륨통로개방제와의 상호작용을 관찰하기 위하여 다음과 같은 실험을 하였다. 체중 20~30 g의 개를 사혈 희생시켜 두개골을 절개한 후 뇌저동맥과 중뇌동맥을 적출하였다. 적출된 동맥편은 4℃의 생리적식염수 내에서 나선형 절편으로 만들어 0.3%의 CHAPS 용액에 침잠시킴으로써 내피세포층을 제거한 후 95% O_2와 5% CO_2의 혼합기체로 포화된 37℃의 Krebs-Henseleit 용액을 포함한 적출근편실험조에서 등척성 장력을 측정하였다.

1. PP, PYY 및 NPY는 뇌동맥 나선형절편을 농도의존적으로 수축시켰으며, 그 효력과 효능은 PYY가 가장 강하였고, 그 다음이 NPY, 그리고 PP의 순이었다. 이들의 효력은 노르아드레날린보다 20내지 200배 강하였으며, 그 중 PYY는 5-HT 보다도 강한 효력을 보였다.

2. Cyclic AMP 활성제인 forskolin과 cyclic GMP 활성제인 sodium nitroprusside는 뇌동맥절편의 기본장력을 감소시켰으며, PP, PYY 및 NPY 유발수축 역시 농도의존적으로 억제하였다. 이 때 forskolin의 기본장력억제작용이 sodium nitroprusside보다 강한 효력을 나타내었다.

3. 칼륨통로 개방제인 RP 49356, P 1060 및 BRL 38227은 기본장력에 대해서는 공히 농도의존적으로 억제하였으나, PP, PYY 및 NPY 유발수축에 대해서는 P 1060만이 농도의존적으로 억제하였고, RP 49356 및 BRL 38227은 약간 억제하는 경향만을 보였는데, 특기할 것은 저농도의(0.1 μM) BRL 38227이 이들 펩타이드 유발수축을 오히려 증가 시켰다는 것이다.

4. 기본장력에 대해서, 칼륨통로개방제들은 forskolin의 이완작용에 유의한 영향을 미치지 못하였으나, 그중 P 1060과 BRL 38227은 sodium nitroprusside의 이완작용을 상승적으로 강화하였다. PYY(0.1 μM)유발 수축작용에 대해서, 칼륨통로 개방제들은 forskolin의 수축억제작용에 대해서는 약간 길항하는 경향만을 보였고, sodium nitroprusside의 수축억제작용은 유의하게 길항하였다.

이상의 결과들을 종합하면, 개의 뇌혈관에서는 NPY 뿐만 아니라 PYY도 혈관수축기전에 중요한 역할을 한다고 볼 수 있으며, 그 수축작용의 기전에는 세포내 cAMP 및 cGMP 활성도의 변화가 포함된다고 사료된다. 또 칼륨통로개방제들은 pancreatic polypeptide family의 뇌혈관 수축작용에 대하여 제제 및 농도에 따라 다양한 영향을 미치므로 이에 대한 향후의 더욱 세밀한 연구가 요구된다.