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Release of Vasoactive Intestinal Peptide in Mouse Cerebral Cortex: Evidence for a Role of Arachidonic Acid Metabolites

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In rodent cerebral cortex, vasoactive intestinal peptide (VIP) is contained in a homogeneous population of radially oriented bipolar interneurons. We have observed that 4-aminopyridine (4-AP), a K^+-channel blocker, promotes a concentration- and Ca^{2+}-dependent release of VIP from mouse cerebral cortical slices, with a significant effect already observed at 50 μM. Over 70% of VIP release elicited by 4-AP is blocked by 2 μM tetrodotoxin (TTX). Mepacrine, an inhibitor of phospholipase A_2 (PLA_2) activity and hence of arachidonic acid (AA) formation from membrane phospholipids, markedly inhibits (IC50 of approximately 15 μM) the release of VIP evoked by 4-AP. The inhibitory effect of mepacrine is not additive to that of TTX, thus indicating an involvement of PLA_2 activation in the TTX-sensitive component of the 4-AP-evoked release. As a corollary, melittin (0.1–10 μg/ml), a PLA_2 activator, promotes VIP release. Inhibition of AA metabolites of the lipoxygenase pathway by nordihydroguaiaretic acid, 5,8,11,14-eicosatetranoic acid, and caffeic acid results in a concentration-dependent inhibition of VIP release evoked by 4-AP.

This set of observations indicates for the first time that the formation of AA metabolites of the lipoxygenase pathway plays a role in the release of a peptide in the mammalian CNS. Furthermore, these observations together with the previously reported potentiation by prostaglandins of the increase in cyclic AMP elicited by VIP in mouse cerebral cortex (Schaad et al., 1987) indicate that AA metabolites may act at both the presynaptic (lipoxygenase metabolites) and the postsynaptic (cyclooxygenase metabolites) levels to increase the "throughput" or "strength" of VIP-containing circuits in the rodent neocortex.

Vasoactive intestinal peptide (VIP) is a 28-amino-acid polypeptide originally isolated from porcine duodenum by Said and Mutt (1970). VIP immunoreactivity has subsequently been demonstrated outside the gastrointestinal tract, including the PNS and the CNS (Larsson et al., 1976; Lorén et al., 1979). In the rodent CNS, VIP is highly concentrated in the cerebral cortex, where it is contained in a homogeneous population of intracortical and radially oriented bipolar neurons (Lorén et al. 1979; Morrison et al., 1984). In the cerebral cortex VIP exerts several cellular actions such as the stimulation of cyclic AMP (cAMP) formation (Quik et al., 1978; Magistretti et al., 1984) and of glycogenolysis (Magistretti et al., 1981) as well as alterations in the spontaneous firing rate of identified neurons (Phillis et al., 1978; Ferron et al., 1985). VIP interacts synergistically with norepinephrine to increase cAMP levels in mouse cerebral cortex (Magistretti and Schorderet, 1984, 1985). Recent evidence indicates that this synergism is mediated by prostaglandins (Schaad et al., 1987). The release of VIP can be evoked by electrical and K^+-evoked depolarizations in a Ca^{2+}-dependent manner (Besson et al., 1982; Wang et al., 1983, 1985). In an attempt to characterize further the position of VIP-containing neurons in cerebral cortical circuitry and their presynaptic regulation, we have examined the molecular mechanisms that underlie the release of VIP evoked by the organic cation 4-aminopyridine (4-AP).

4-AP is a blocker of 2 voltage-sensitive K^+ currents, the delayed rectifier and the A-current, a transient outward current (Thompson, 1977; Gustafsson et al., 1982; Segal et al., 1984; Rogawski, 1985). Blockade of the delayed voltage-sensitive K^+ channel prolongs the action potential at nerve terminals by inhibiting the repolarization phase. This action results in an enhancement of neurotransmitter release because of the increase in the open time of voltage-sensitive calcium channels in the nerve terminal membrane (Lemeignan, 1972; Jankowska et al., 1977; Heuser et al., 1979; Thesleff, 1980). A-currents play an important role in controlling excitability at voltages near the resting potential (Rogawski, 1985) by preventing small depolarizing stimuli to reach threshold and trigger action potentials. Therefore, by blocking voltage-sensitive K^+ channels, 4-AP may also increase the inflow of Ca^{2+} into nerve terminals and stimulate neurotransmitter release. Finally, evidence has been presented for a direct effect of 4-AP on Ca^{2+} channels, favoring Ca^{2+} inflow (Lundh and Thesleff, 1977; Illes and Thesleff, 1978; Rogawski and Barker, 1983).

In the present set of investigations we have examined the release of VIP from mouse cerebral cortical slices elicited by 4-AP and have observed that (1) 4-AP promotes a concentration-dependent release of VIP in a Ca^{2+}-dependent manner, (2) over 70% of 4-AP-evoked VIP release is blocked by tetrodotoxin (TTX), and (3) the TTX-sensitive component of VIP release evoked by 4-AP is mediated by arachidonic acid (AA) metabolites of the lipoxygenase pathway.

Materials and Methods

Release experiments. Swiss male albino mice were used throughout this study. They were maintained in an alternating 12 hr light/12 hr dark

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cycle and had free access to food and water. Cerebral cortical slices were prepared as follows (Magistretti et al., 1981). Mice were decapitated and their brains rapidly removed. The cerebral cortex was then dissected on ice and immediately placed in a modified Krebs-Ringer bicarbonate buffer, pH 7.4 (KRG) containing (in mM concentrations): NaCl, 120; KCl, 3; $CaCl_2$, 2.6; $MgSO_4$, 0.67; KH_2PO_4, 1.2; glucose, 3; $NaHCO_3$, 27.5, previously gassed with O_2/CO_2 (95:5). The dissected cortices (1 cortex for approximately 8 tubes) were then placed on a McIlwain tissue chopper, their ventral aspect facing the chopping plate, and 250 μm slices were prepared. The plate was then rotated by 90°, and the cortices were cut again. The slices were then resuspended (using a glass pipette) in KRG and incubated for 90 min at 37°C under continuous gassing (O_2:CO_2, 95:5) and vigorous shaking. The medium was replaced every 30 min with fresh KRG. At 90 min, after the last medium replacement, 450 μl of the slice suspension (1–2 mg of proteins) were distributed into Eppendorf tubes. Drugs (25 μl) were then added for 4 min (4-AP and K^+). The inhibitors were added 2 min prior to the depolarizing agents. At the end of the incubation, the tubes were centrifuged for 2 min at 9980 × g; an aliquot of the supernatant was removed, lyophilized in a Speed-Vacuum concentrator (Savant Instrument), and resuspended in radioimmunoassay (RIA) buffer. VIP released in the supernatant was measured by RIA as described by Martin et al. (1986). The remaining supernatant was removed, and 750 μl of 1 M CH_3COOH were added to the pellet for extraction of VIP from tissue. The solution was boiled for 15 min and sonicated. Aliquots of 20 μl were tested for protein concentration as described by Lowry et al. (1951). The tubes were then centrifuged for 2 min (9980 × g), an aliquot of the supernatant was diluted and lyophilized, and VIP tissue content was measured by RIA (Martin et al., 1986). For the RIA procedure, samples were incubated for 2 d at 4°C using a well-characterized molecular species of ^{125}IVIP, i.e., (mono(^{125}I)iodo-Tyr^{10},MetO^{17})-VIP, which is monoiodinated on Tyr 10 and oxidized on Met 17 (Martin et al., 1986). The lower limit of detection of this RIA is 3 pg. No cross-reactivity is observed either with other peptides sharing structural homologies with VIP, such as peptide having N-terminal histidine and C-terminal isoleucine amide (PHI), secretin 5-27, glucagon, GRF 1-44 NH_2, or GRF 1-28 OH, or with peptides unrelated to VIP, such as somatostatin (SS-14), neurotensin, bombesin, leu-enkephalin, or met-enkephalin. The intra-assay variation of the RIA is 8%, and the interassay variation 10.6%. Statistical analysis was performed using the Student's *t*-test.

VIP was purchased from Professor V. Mutt, Department of Biochemistry II, Karolinska Institute, Stockholm. Rabbit anti-VIP antiserum was from Amersham International. Caffeic acid, indomethacin, melittin, and mepacrine were purchased from Sigma (St. Louis, MO), 4-AP from Merck (Darmstadt, FRG), and 5,8,11,14-eicosatetraenoic acid (ETYA), 5(S)-hydroperoxyeicosatetraenoic acid (HPETE), 12(S)-HPETE, and TTX from Calbiochem (La Jolla, CA).

Reverse-phase high-performance liquid chromatography analysis of VIP extracts. Tissue acid extracts (1 M CH_3COOH) containing VIP were further purified through a Sep-pack C_{18} cartridge previously washed with 5 ml of MeOH and pre-equilibrated with 10 ml of 10% CH_3CN vol/vol in 1% TFA–tetraethylammonium (TEA) (pH 2.5). The column was then washed with 5 × 2 ml of 10% CH_3CN in 1% TFA-TEA (pH 2.5), and VIP was eluted with 2 ml of 60% CH_3CN in 1% TFA-TEA (pH 2.5). The volume of the eluted product was reduced under N_2 flux and then passed through a 0.45 μm Millipore column. Further purification of the product was performed on a Waters reverse-phase high-performance liquid chromatography (RP-HPLC) system using a Macherey-Nagel C_8 column (5 μm diameter, 300 Å pore size, end capped 0.4 × 25 cm). The equilibrated column was first eluted isocratically with 75% of 0.1% TFA and 25% of 90% CH_3CN (in 0.1% TFA) at a flow rate of 0.6 ml/min. After 5 min, a linear gradient of 90% CH_3CN (in 0.1% TFA) was started, 25% to 45% over 40 min. Porcine VIP was detected at 214 nm. The samples were collected in 100 μl of 1 mg/ml BSA and then lyophilized and reconstituted in 50 mM Tris-HCl buffer, pH 7.4, containing 5 mM $MgCl_2$, 2 mM EGTA, 0.2% BSA, and 0.1 mM bacitracin. VIP-like immunoreactivity in an aliquot of each fraction was assayed by RIA.

Results

An RP-HPLC procedure was used to characterize VIP-like immunoreactivity (VIP-LI) extracted from mouse cerebral cortical slices using conditions described in Materials and Methods. The RP-HPLC purification followed by determination of VIP-LI tissue content of each fraction by RIA shows a single peak eluting at a retention time of 37 min (Fig. 1). The position (on the same gradient) of native VIP monitored for absorbance at 214 nm corresponds to 37 min. No difference between the elution position of native VIP and VIP-LI extracted from cerebral cortex was observed. The short tail appearing at 42 min may be explained either by the presence in the extract (at the C-terminal) of desamidated VIP, which is expected to elute at a longer retention time than does native VIP, or by the rather small quantity (3–4 ng) of VIP passed through the column.

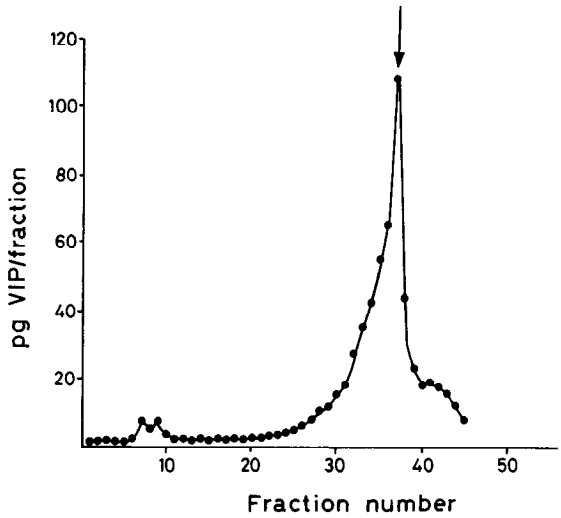

Figure 1. RP-HPLC characterization of VIP tissue content extracts. Mouse cerebral cortical slices corresponding to a protein concentration of 1.78 mg were extracted, chromatographed on a Sep-pack C_{18} cartridge, and passed through a 0.45 μm Millipore column. The extracted product was the analyzed by RP-HPLC as described in Materials and Methods. Fractions of 0.6ml/min were collected in 100 μl of 1 mg/ml BSA, lyophilized, and assayed for their VIP-LI content by RIA (Martin et al., 1986). The elution position of native VIP, determined by absorbance at 214 nm, is indicated by the *arrow* and corresponds to 37 min.

The release of VIP evoked by 4-AP was examined. As shown in Figure 2, 4-AP promotes a concentration-dependent release of VIP, with a significant effect already observed at 50 μM. At 1 mM, 4-AP increases by almost 6-fold the basal release of VIP. We have also observed that 4-AP enhances the release of VIP elicited by small depolarizing stimuli, such as those achieved by raising the extracellular K^+ concentration to 7 or 10 mM (Table 1). These K^+ concentrations are ineffective (7 mM) or marginally effective (10 mM) per se in stimulating VIP release (Table 1).

The 4-AP-evoked release of VIP is Ca^{2+}-dependent, as indicated by the complete block observed after addition to the medium of the Ca^{2+} channel blocker Co^{2+} at a concentration of 5 mM: basal release (% of VIP content) = 0.232 ± 0.02; 4-AP (1 mM) = 1.7 ± 0.07; and 4-AP (1 mM) + Co^{2+} (5 mM) = 0.221 ± 0.02. Mn^{2+}, another inorganic Ca^{2+} channel blocker, also significantly inhibited (by 69% at 1 mM) the release of VIP evoked by 4-AP: basal release (% of VIP content) = 0.223 ± 0.03; 4-AP (1 mM) = 1.11 ± 0.07; and 4-AP (1 mM) + Mn^{2+} (1 mM) = 0.499 + 0.02.

The release of VIP evoked by 4-AP most likely results from an increase in impulse activity in the slices. However, depolarization of VIP-containing nerve terminals associated with regenerative Ca^{2+} currents cannot be excluded (Llinas et al., 1976; Lundh and Thesleff, 1977; Molgo et al., 1980; Dolezal and

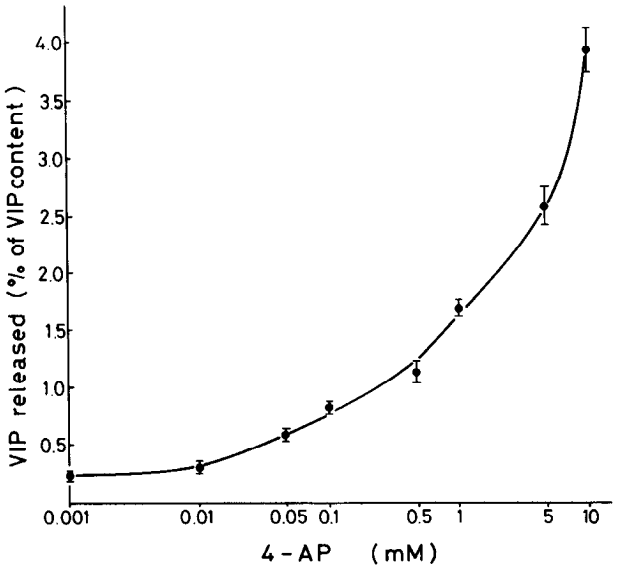

Figure 2. Effect of increasing concentrations of 4-AP on basal VIP release. Mouse cerebral cortical slices were incubated as described in Materials and Methods. VIP released is expressed as percentages of VIP tissue content. Results are the means ± SEM of 4 determinations in each of 2 separate experiments, except for basal VIP release (n = 8 in each experiment). Basal VIP release was 0.301 ± 0.021%; n = 16. Absolute values of VIP released into the medium and of VIP content were 11.99 ± 0.75 pg/mg prot (n = 16) and 4050.48 ± 158.23 pg/mg prot (n = 16), respectively.

Tucek, 1983). In an attempt to determine the relative contribution of these 2 mechanisms, we examined the effect of TTX, a blocker of voltage-sensitive Na^+ channels on the release of VIP evoked by 4-AP. As indicated in Figure 3, TTX at 2 μM, a concentration previously shown to completely block voltage-sensitive Na^+ channels (Moore et al., 1967; Ulbricht, 1974; Alvarez-Leefmans and Miledi, 1980; Dolezal and Tucek, 1983) and therefore the propagation of action potentials, markedly inhibits the release of VIP induced by 4-AP (73.97 ± 1.71%; n = 8). This observation suggests that approximately 70% of VIP release is dependent on the presence of action potentials, whereas 30% of the release may reflect a direct action of 4-AP on VIP terminals.

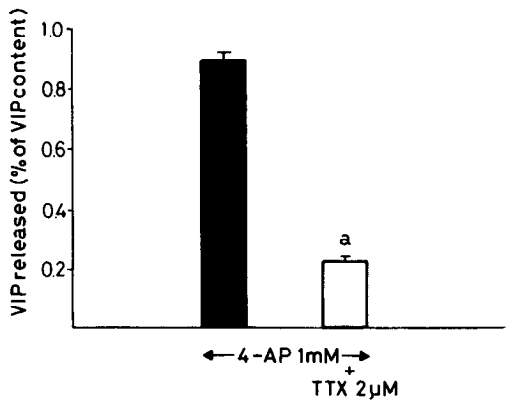

Figure 3. Inhibition by TTX of 4-AP evoked release of VIP. Mouse cerebral cortical slices were incubated as described in Materials and Methods. These results are the means ± SEM of 7–8 determinations in each of 2 separate experiments. Basal VIP release was 0.208 ± 0.013% (n = 7). Absolute values of VIP released into the medium and of VIP content were 8 ± 0.56 pg/mg prot (n = 7) and 3841.43 ± 99.31 pg/mg prot (n = 7), respectively. No effect on basal VIP release was observed with 2 μM TTX, e.g.: basal VIP release = 0.309 ± 0.034% (n = 4); TTX (2 μM) alone = 0.277 ± 0.013% (n = 4). *a*, Significantly different from 4-AP-induced VIP released ($p < 0.0005$).

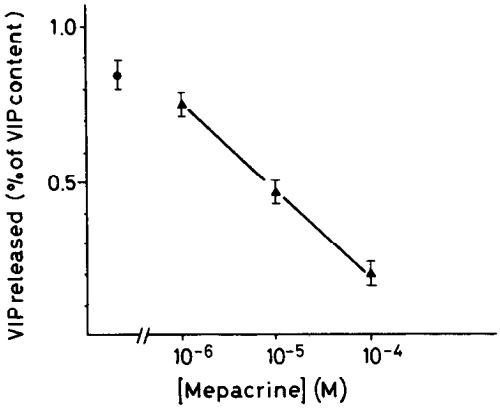

Figure 4. Concentration–response curve of the inhibition by mepacrine of 4-AP-evoked VIP release. Mouse cerebral cortical slices were incubated as described in Materials and Methods. Results are the means ± SEM of 6 determinations from one experiment, repeated once with similar results. These results are expressed as percentages of VIP tissue content. Basal VIP release was 0.201 ± 0.022%, n = 7. Absolute values of VIP released into the medium and of VIP content were 7.24 ± 0.096 pg/mg prot (n = 7) and 3544.8 ± 139 pg/mg prot (n = 7), respectively. Mepacrine (50 μM) did not affect basal VIP release, e.g.: basal VIP release = 0.267 ± 0.013% (n = 5); mepacrine (50 μM) alone = 0.262 ± 0.012% (n = 6).

In a series of experiments in which we set out to examine the molecular mechanism(s) involved in the release of VIP, we made the observation that mepacrine, an inhibitor of phospholipase A_2 (PLA_2) activity, inhibited in a concentration-dependent manner the release of VIP evoked by 4-AP, with an IC_{50} of 15 μM (Fig. 4), without affecting basal release (legend for Fig. 4). Mepacrine, by inhibiting PLA_2 activity, impairs the formation of AA and hence of its metabolites (Irvine, 1982). This observation indicates that inhibition of PLA_2 activity and of AA formation interferes with the expression of the release process. It should, however, be noted that mepacrine can act on other target enzymes (Chang et al., 1987). In order to further pinpoint an involvement of PLA_2 in the release of VIP, we tested melittin,

Table 1. Effect of 4-AP (1 mM) on basal and K^+-evoked release of VIP

Agent(s) added	Concentrations (mM)	VIP released (% of VIP content)
None	—	0.326 ± 0.017
4-AP	1	1.413 ± 0.12[a]
K^+	7	0.273 ± 0.017
+4-AP	1	1.71 ± 0.155[b]
K^+	10	0.438 ± 0.03
+4-AP	1	2.975 ± 0.278[c]

Mouse cerebral cortical slices were incubated as described in Materials and Methods. K^+ concentration under basal conditions was 3 mM. VIP released is expressed as percentage of VIP tissue content. Results are the means ± SEM of 4–12 separate determinations. Absolute values of VIP released into the medium and of VIP content were 9.68 ± 0.33 pg/mg prot (n = 12) and 3016.88 ± 116.67 pg/mg prot (n = 12).

[a] Significantly different from basal VIP release ($p < 0.0005$).

[b] Significantly different from VIP release elicited by K^+ (7 mM) ($p < 0.0005$).

[c] Significantly different from VIP release elicited by K^+ (10 mM) ($p < 0.0005$).

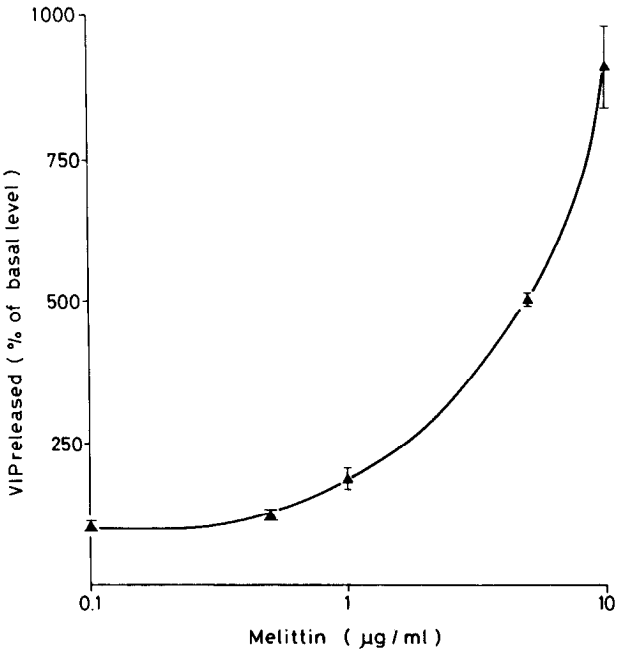

Figure 5. Effect of increasing concentrations of melittin on basal VIP release. Mouse cerebral cortical slices were incubated as described in Materials and Methods. Results are the means ± SEM of 4 determinations from 2 separate experiments. These results are expressed as percentages of VIP tissue content. Basal VIP release was 0.257 ± 0.021%, $n = 7$. Absolute values of VIP released into the medium and of VIP content were 10.6 ± 1.29 pg/mg prot ($n = 7$) and 4092.11 ± 281.72 pg/mg prot ($n = 7$), respectively. No cross-reactivity was observed in the radioimmunoassay with melittin at a concentration of 10 µg/ml.

an activator of PLA_2 (Shier, 1979). As shown in Figure 5, melittin promotes a concentration-dependent release of VIP, with significant effects already observed at 1 µg/ml.

Through the action of the enzyme, cyclooxygenase prostaglandins and thromboxane are formed from AA, whereas lipoxygenase activity yields leukotrienes from AA. Three inhibitors of lipoxygenase activity, namely ETYA, nordihydroguaiaretic acid (NDGA), and caffeic acid, inhibited in a concentration-dependent manner the release of VIP evoked by 4-AP (Fig. 6 *A, B, C*), with IC_{50} of 45 µM, 50 µM, and 1.5 mM, respectively. Indomethacin, a cyclooxygenase inhibitor (Vane, 1971) that displays at µM concentrations an inhibitory activity towards lipoxygenase (Salmon, 1986; Marshall, 1988), exerted at 100 µM a moderate but significant inhibition of VIP release

Table 2. Lack of effect of mepacrine and caffeic acid on the K^+ evoked release of VIP

Agent(s) added	Concentration (mM)	VIP released (% of VIP content)
None	—	0.227 ± 0.016
K^+	20	1.327 ± 0.089
+ Mepacrine	0.05	1.342 ± 0.032[a]
+ Caffeic acid	1	1.417 ± 0.103[a]

Experimental conditions as in Figures 2–4 and Table 1, described in detail in Materials and Methods. Results are the means ± SEM of 3–9 determinations from 2 separate experiments and are expressed as percentages of VIP tissue content. Absolute values of VIP released into the medium and of VIP content were 7.47 ± 0.56 pg/mg prot ($n = 9$) and 3283.68 ± 85.52 pg/mg prot ($n = 9$).

[a] Not significantly different from VIP release evoked by K^+ (20 mM) ($p > 0.05$).

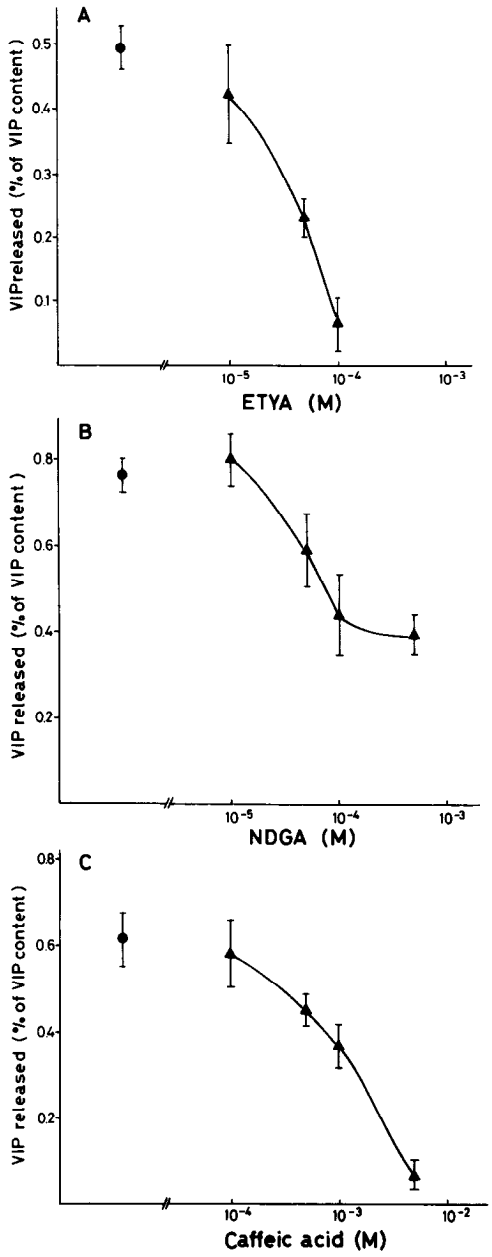

Figure 6. Effect of various lipoxygenase inhibitors, i.e., ETYA, NDGA, and caffeic acid on 4-AP evoked release of VIP. Mouse cerebral cortical slices were incubated as described in Materials and Methods. Results are the means ± SEM of 4 determinations and are expressed as percentages of VIP tissue content. *A*, Inhibitory effect of ETYA. Basal VIP release was 0.644 ± 0.049% ($n = 4$). Absolute values of VIP released into the medium and of VIP content were 20.64 ± 0.67 pg/mg prot ($n = 4$) and 3243.04 ± 183.44 pg/mg prot ($n = 4$), respectively. *B*, Inhibitory effect of NDGA. Basal VIP release was 0.32 ± 0.01% ($n = 4$). Absolute values of VIP released into the medium and of VIP content were 18.36 ± 1.37 pg/mg prot ($n = 4$) and 5721.77 ± 335.98 pg/mg prot ($n = 4$), respectively. *C*, Inhibitory effect of caffeic acid. Basal VIP release was 0.358 ± 0.034% ($n = 4$). Absolute values of VIP released into the medium and of VIP content were 18.46 ± 1.83 pg/mg prot ($n = 4$) and 5144.54 ± 78.82 pg/mg prot ($n = 4$) respectively.

evoked by 4-AP. This effect, however, was only marginally additive to that of caffeic acid: basal release (% of VIP content) = 0.3 ± 0.03; 4-AP (1 mM) = 1.55 ± 0.04; 4-AP (1 mM) + indomethacin (100 µM) = 1.08 ± 0.1; 4-AP (1 mM) + caffeic acid (500 µM) = 0.96 ± 0.03; and 4-AP 1 (mM) + indomethacin

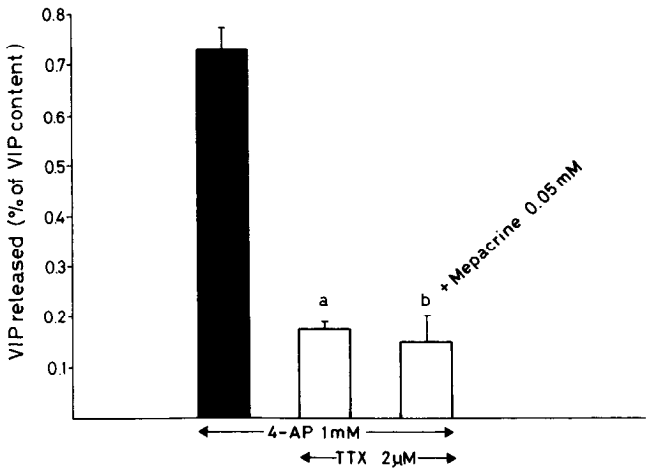

Figure 7. Lack of additive effect of mepacrine on 4-AP-evoked VIP release in the presence of TTX (2 μM). Mouse cerebral cortical slices were incubated as described in Materials and Methods. Results are the means ± SEM of 6–8 determinations from 2 experiments and are expressed as percentages of VIP tissue content. Basal VIP release was 0.209 ± 0.023% (n = 8). Absolute values of VIP released into the medium and of VIP content were 8.02 ± 0.76 pg/mg prot (n = 8) and 3900.31 ± 141 pg/mg prot (n = 8), respectively. *a*, Significantly different from 4-AP- evoked VIP release ($p < 0.0005$). *b*, Not significantly different from 4-AP-evoked VIP release in presence of TTX ($p > 0.05$).

(100 μM) + caffeic acid (500 μM) = 0.84 ± 0.02. It is worth noting that mepacrine (50 μM) and caffeic acid (1 mM) did not inhibit the K^+-evoked VIP release (Table 2).

In an attempt to identify the lipoxygenase metabolites involved in the release of VIP evoked by 4-AP, we examined the effect of 5(S)-HPETE and 12(S)-HPETE on basal VIP release. These 2 compounds were, however, without effect (data not shown).

In view of the foregoing observations, we set out to determine whether the inhibition of AA metabolites formation affected preferentially the TTX-sensitive or -insensitive components of the 4-AP-evoked VIP release. Figure 7 shows that the inhibitory effect of mepacrine is not additive to TTX, thus indicating an action on the TTX-sensitive component.

Discussion

In the series of experiments reported in this article, we have observed that 4-AP promotes a concentration-dependent release of VIP in a Ca^{2+}-sensitive manner. Over 70% of VIP release elicited by 4-AP is TTX-sensitive. This TTX-sensitive component is mediated by AA metabolites. TTX at 1 μM was previously shown to decrease (by 60–68%) the release of ACh elicited by 0.1 mM 4-AP from rat striatal slices (Dolezal and Tucek, 1983). More recently, Damsma et al. (1988) observed that TTX at 1 μM completely blocked the 4-AP (0.1 mM)-induced release of ACh from striatum of freely moving rats (Damsma et al., 1988).

The existence of a conspicuous component of the 4-AP-evoked VIP release sensitive to TTX indicates that 4-AP increases the occurrence of activatory inputs onto VIP neurons. The basal impulse activity in the slices appears to be quite moderate in view of the absence of inhibitory effect of TTX on basal VIP release (see legend for Fig. 3). However, the TTX sensitivity of the 4-AP-evoked VIP release may in fact reflect the inhibition of the release of one (or more) neurotransmitter(s), from terminals impinging on VIP-containing neurons; such neurotransmitter(s) could in turn promote the release of VIP through a receptor-mediated mechanism. The observation that the TTX-sensitive 4-AP-evoked VIP release is inhibited by agents that block the formation of AA metabolites would support this view, since PLA_2 may be activated by receptor-mediated mechanisms (Burch et al., 1986a, b; Axelrod et al., 1988). Furthermore, AA metabolites of the lipoxygenase pathways have recently been shown to regulate presynaptic activity and neurotransmitter release (Piomelli et al., 1987a, b). Thus, in *Aplysia* sensory neurons the synaptic actions of the neuroactive peptide FMRFamide, which produces presynaptic inhibition of transmitter release, are mediated by lipoxygenase metabolites of AA. This mechanism is supported by observations indicating that membrane hyperpolarization elicited by FMRFamide is abolished after injection of NDGA, a lipoxygenase inhibitor. Furthermore, the response to FMRFamide can be mimicked by the lipoxygenase metabolite 12-HPETE (Piomelli et al., 1987a). In another report, Piomelli and colleagues (1987b) reported that in *Aplysia* the application of histamine and stimulation of the histaminergic neuron C2 or neurons in the L32 cluster promote the formation of lipoxygenase products of AA, possibly through activation of postsynaptic receptors that are linked to a phospholipase. These results indicate that lipoxygenase metabolites may be second messengers for presynaptic inhibition of these neurons. While supporting a role of AA metabolites in presynaptic release in the mammalian CNS, results reported in the present article, indicate that lipoxygenase products of AA would mediate the stimulation rather than the inhibition of neurotransmitter release in mouse neocortex.

Interestingly, in contrast to what is observed with 4-AP, the K^+-evoked release of VIP does not appear to involve AA metabolites, since no effect of mepacrine or caffeic acid is observed (Table 2). A major difference between K^+ and 4-AP-evoked VIP release is that the former is not blocked by TTX (Besson et al., 1982; Martin and Magistretti, in press).

In summary, our observations indicate that 4-AP promotes the release of VIP, in part (approximately 30%) via a direct action on VIP-containing terminals, possibly by activating regenerative Ca^{2+} currents at this level, and in part (approximately 70%) by eliciting the release of one (or more) neurotransmitter(s) that would stimulate VIP release through a PLA_2-dependent mechanism.

4-AP is known to block at least 2 types of voltage-sensitive K^+ channels, the delayed rectifier and the A-current (Thompson, 1977; Gustafsson et al., 1982; Hille, 1984; Segal et al., 1984; Rogawski, 1985). Interestingly, actions on the latter current are exerted at μM concentrations of 4-AP, whereas at mM concentrations blockade of the delayed rectifier is apparent (Thompson, 1977; Rogawski, 1985). The concentration–response curve of the release of VIP (Fig. 2) is therefore consistent with an action of 4-AP on A-currents. This view is further supported by 2 sets of observations. First, TEA, which potently blocks the delayed rectifier but considerably less so the A-currents, promotes a marginal release of VIP [VIP release, in % of content: basal = 0.243 ± 0.014; TEA (10 mM) = 0.398 ± 0.046]. Second, analysis of the kinetics of A-currents had indicated that they activate very rapidly following depolarizations ranging from −60 to −45 mV (Connor and Stevens, 1971; Neher, 1971); they also inactivate rapidly with maintained depolarization. Owing to these properties, A-currents briefly repolarize (because of their rapid kinetics) the membrane potential following small depolar-

izing stimuli. As shown in Table 1, 4-AP reveals a releasing action of otherwise marginally effective K^+ concentrations (i.e., small depolarizing stimuli). This observation is consistent with the view that in the absence of 4-AP the depolarization elicited by low K^+ concentrations and the subsequent Ca^{2+}-dependent VIP release are dampened by the activation of transient outward A-currents. Blockade of these currents by 4-AP would remove this shunt and reveal the releasing effect of low K^+ concentrations. Similar observations have been made for the glycogenolytic effect of low K^+ concentrations, which is considerably enhanced by 4-AP in mouse cerebral cortical slices (Hof et al., 1988).

Finally, the possibility should be considered that at least part of both TTX-sensitive and TTX-insensitive 4-AP-evoked VIP release may be the consequence of a direct effect of the aminopyridine on voltage-sensitive Ca^{2+} inflow into presynaptic nerve terminals, which would be independent on the blockade of outward K^+ currents (Lundh and Thesleff, 1977; Illes and Thesleff, 1978; Rogawski and Barker, 1983).

In the rodent neocortex, VIP is contained in bipolar and radially oriented interneurons that branch only minimally in the horizontal plane (Connor and Peters, 1984; Morrison et al., 1984). These morphological characteristics imply that the released VIP molecules will exert their actions locally, within radially restricted cortical domains or columns (Magistretti and Morrison, 1988). Interestingly, it was recently demonstrated that AA metabolites, and more specifically the prostaglandins E_2 and $F_{2\alpha}$, strongly potentiate the increases in cAMP levels elicited by VIP in mouse cerebral cortical slices (Schaad et al., 1987). This potentiation most likely represents the molecular mechanism of the synergistic interaction between VIP and noradrenaline in stimulating cAMP formation (Magistretti and Schorderet, 1984, 1985). Taken together, these observations suggest that AA metabolites may play a role in the modulation of VIP-mediated neurotransmission at both presynaptic and postsynaptic sites.

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