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Stimulation by Glutamate Receptors of Arachidonic Acid Release Depends on the Na^+/Ca^{2+} Exchanger in Neuronal Cells

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SUMMARY

In primary cultures of striatal neurons, stimulation of *N*-methyl-D-aspartic acid (NMDA) receptors or associative activation (but not separate activation) of (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and metabotropic glutamate receptors (mGluR) strongly increased arachidonic acid (AA) release via activation of phospholipase A_2 (PLA_2). Depolarizing agents, such as veratridine, were as potent as NMDA in stimulating AA release. However, increasing the intracellular Ca^{2+} concentration via voltage-sensitive Ca^{2+} channels did not result in a significant stimulation of PLA_2. Substitution of sodium by lithium, a monovalent cation that does not participate in the Na^+/Ca^{2+} exchanger activity but permeates ionotropic glutamate receptor channels, blocked AA release induced by veratridine or AMPA plus mGluR agonists. It also reduced the NMDA-induced AA release, to a lesser extent. The contribution of the Na^+/Ca^{2+} exchanger to the activation of PLA_2 after veratridine, NMDA receptor, or AMPA receptor plus mGluR stimulation was confirmed by using a selective inhibitor of the Na^+/Ca^{2+}exchanger.

Glu, like other neurotransmitters, activates channel receptors (ionotropic receptors) and receptors coupled to guanine nucleotide-binding proteins (metabotropic receptors) (1–4). Three families of ionotropic Glu receptors have already been described, the NMDA, AMPA, and KAI receptors. KAI and AMPA receptor channels are permeable to monovalent cations (Na^+ and K^+) and increase $[Ca^{2+}]_i$ mainly via depolarization-induced opening of VSCC (1, 5). A minority of AMPA/KAI receptors are permeable to Ca^{2+} (6). In contrast to most KAI and AMPA receptors, NMDA receptor channels appear to be largely permeable to Ca^{2+} ions, in addition to Na^+ and K^+ ions (7). One class of metabotropic receptors activates phospholipase C and thus inositol trisphosphate formation, as well as intracellular Ca^{2+} release (3, 6, 8–12).

In addition to these primary ionic and enzymatic events, at least two other messengers have been associated with Glu receptor activation, namely AA and NO (13–15). A third, cGMP, is also produced as a consequence of guanylate cyclase activation by NO (15–20). AA and NO are interesting messengers because they can easily diffuse in and out of the cell and therefore behave as intra- and intercellular messengers. Therefore, in addition to acting within the cells where they are produced, there is evidence that they can also act as retrograde messengers by transducing information from the postsynaptic to the presynaptic element (21–27). Such a function appears to be of crucial importance in long term potentiation, which is likely to represent an elementary event underlying learning and memory (28, 29).

We have demonstrated that AA is produced in striatal neurons by combined stimulation of AMPA receptors and either mGluR or NMDA receptors (13, 14). The requirement of a combined stimulation of AMPA and NMDA receptors for releasing AA can be easily explained. Indeed, NMDA receptor channels are blocked by Mg^{2+} ions at resting membrane potential and AMPA-induced depolarization is required to suppress the Mg^{2+} blockage (30, 31). It is believed that, once activated, NMDA receptor channels lead to sufficient Ca^{2+} entry to stimulate phospholipase A_2 (7). However, we have no indication as to the molecular mechanisms underlying the associative stimulation of AMPA receptors and mGluR leading to AA release (14). In this report, we provide evidence for a crucial role of Ca^{2+} influx via the Na^+/Ca^{2+} exchanger in this associative process.

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ABBREVIATIONS: Glu, glutamate; NMDA, *N*-methyl-D-aspartic acid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; KAI, kainic acid; QA, quisqualic acid; *t*-ACPD, (*trans*)-1-amino-cyclopentyl-1,3-dicarboxylic acid; AA, arachidonic acid; CBDMB, 5-(*N*-4-chlorobenzyl)-2′,4′-dimethylbenzamil; MGCMA, 5-[*N*-methyl-*N*-(guanidinocarbonylmethyl)]amiloride; TTX, tetrodotoxin; VSCC, voltage-sensitive Ca^{2+} channels; PTX, *Bordetella pertussis* toxin; NO, nitric oxide; HEPES, *N*-[α-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; mGluR, metabotropic glutamate receptor(s); PKC, protein kinase C; $[Ca^{2+}]_i$, intracellular calcium concentration; $[Na^+]_i$, intracellular sodium concentration.

Materials and Methods

Primary neuronal cultures. Primary cultures of striatal neurons were prepared as described previously (32). Striata were removed from 14–15-day-old mouse embryos (Iffa Credo, Lyon, France) and cells were gently dissociated in culture medium and plated (1.5×10^6/dish) in 35-mm-diameter Costar (Cambridge, MA) culture dishes (six-well clusters, 2 ml/well). The dishes had been previously coated with poly-L-ornithine (15 μg/ml, M_r 40,000) and culture medium containing 10% fetal calf serum. Culture was then performed in a defined medium (32). Under these conditions, the cultures have been shown to be highly enriched in neurons that form many mature and functional synapses (32). Cultures were maintained at 37° in a humidified atmosphere in 6% CO_2/94% air.

Measurement of [^{3}H]AA release. Striatal neurons, after 13 days *in vitro*, were incubated overnight with 0.25 μCi/ml [^{3}H]AA (0.5 μCi/well; specific activity, 166 Ci/mmol; CEA, Saclay, France). As reported previously (13), >90% of the radioactivity was incorporated into the cells. Eighteen to 24 hr later, cells were washed five times (at 5-min intervals) with 2 ml/well of Krebs bicarbonate buffer (3.5 mM KCl, 0.75 mM $CaCl_2$, 124 mM NaCl, 1.25 mM KH_2PO_4, 26.3 mM $NaHCO_3$, 10 mM glucose, pH 7.4) containing 0.05% bovine serum albumin (fatty acid free); the buffer was saturated with 95% O_2/5% CO_2. Neurons were then exposed to the stimulating medium for 15 min. The radioactivity released in the medium (^{3}H-med) and that remaining in the cells (^{3}H-cell) were determined. The percentage of radioactivity released from the cells was calculated as 100 × [^{3}H-med/(^{3}H-med + ^{3}H-cell)]. For the sodium substitution experiments, the Krebs bicarbonate buffer was replaced with modified Krebs buffer, in which sodium chloride could be totally substituted by equimolar concentrations of lithium chloride (3.5 mM KCl, 0.75 mM $CaCl_2$, 124 mM NaCl or 124 mM LiCl, 1.25 mM $KHPO_4$, 10 mM $KHCO_3$, 10 mM glucose, pH 7.4); 0.05% bovine serum albumin (fatty acid free) was added and the buffer was saturated with 95% O_2/5% CO_2.

Electrophysiological techniques. Recordings were performed at room temperature from 2-week-old cultured neurons, using the whole-cell configuration of the patch-clamp technique. External solution contained 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 0.3 μM TTX, and 10 mM HEPES, pH 7.4. In experiments using NMDA, $MgCl_2$ was omitted and 1 μM glycine was added to the solution. In all cases, internal medium contained 140 mM CsCl, 0.5 mM $CaCl_2$, 5 mM EGTA, and 10 mM HEPES, pH 7.2. Recording pipettes filled with this internal medium had impedance ranging from 3 to 5 MΩ. Drug solutions were adjusted to pH 7.4 and were applied to individual cells using a rapid perfusion system.

Materials. The chemicals used in the present study were obtained from the following sources. NMDA, AMPA, and QA were purchased from Euromedex (Strasbourg, France). Veratridine and TTX were from Sigma (L'Isle d'Abeau, France). *t*-ACPD was from Tocris Neuramin (Essex, England). Staurosporine was from Calbiochem (Meudon, France). [^{3}H]AA (specific radioactivity, 166 Ci/mmol) was from CEA. CBDMB and MGCMA were from Dr. E. J. Cragoe (Nacogdoches, TX).

Results

Evidence that mGluR stimulation must be associated with Na^+ entry to trigger AA release. Separate stimulations of AMPA receptors and mGluR with their respective specific agonists, AMPA and *t*-ACPD, resulted in a weak stimulation of AA release (Fig. 1A). In contrast, combined stimulation of these receptors was very active in triggering AA release (Fig. 1A) (14). In a previous paper (33), we fully characterized the radioactivity released from neuronal cells in culture, after prelabeling with [^{3}H]AA and after drug treatment leading to a large increase in $[Ca^{2+}]_i$ (Glu and ionomycin). Only [^{3}H]AA (and not cyclooxygenase or lipoxygenase derivatives) was detected (33). Because AMPA receptor stimulation is known to

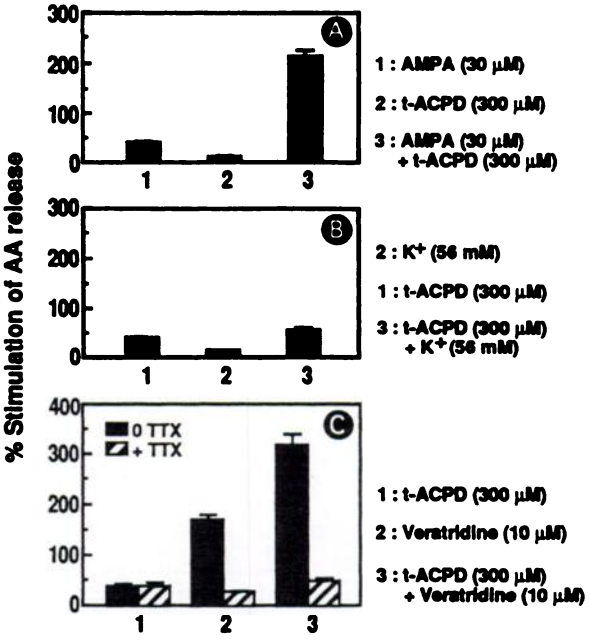

Fig. 1. Sodium dependence of AMPA- plus *t*-ACPD- and veratridine-induced stimulation of AA release. A, Effect of 30 μM AMPA and 300 μM *t*-ACPD, alone or together, on [^{3}H]AA release in striatal neurons after 13 days *in vitro*. B, Effect of neuronal depolarization induced by 56 mM K^+ alone or associated with 300 μM *t*-ACPD on [^{3}H]AA release. C, Effect of neuronal depolarization induced by 10 μM veratridine alone or associated with 300 μM *t*-ACPD in the presence or absence of 0.5 μM TTX. Neurons were exposed to 0.5 μM TTX 20 min before and during the 15-min stimulation period with either 10 μM veratridine, 300 μM *t*-ACPD, or 10 μM veratridine plus 300 μM *t*-ACPD. In A, B, and C, neurons were stimulated with the indicated concentrations of agonists and the percentage of radioactivity released from the cells was calculated as indicated in Materials and Methods. Results are expressed as percentage of stimulation over basal release (basal release, 0.60 ± 0.12% of the total radioactivity in the cells). In histograms, each *bar* is the mean ± standard error of four separate experiments, each performed in triplicate.

increase Na^+ conductance, depolarize neurons, and open VSCC leading to an increase of $[Ca^{2+}]_i$, we tried to determine which of these events triggered AA release when associated with mGluR stimulation. Obviously, depolarization was not a crucial event because K^+ (56 mM) alone or in association with *t*-ACPD application was ineffective in triggering AA release (Fig. 1B). In contrast, veratridine alone (a Na^+ channel opener) was a good stimulator of AA release and was even stronger when associated with mGluR stimulation (Fig. 1C). The effects of veratridine were suppressed by TTX, suggesting that they were due to Na^+ entry via voltage-sensitive Na^+ channels (Fig. 1C). Ouabain (100 μM), an agent that increased $[Na^+]_i$ and depolarized neurons, also induced AA release in combination with mGluR stimulation (120% of basal release) (data not shown).

Requirement for extracellular Ca^{2+} to trigger AA release. As shown in Fig. 2, AA release induced by combined stimulation of AMPA receptors and mGluR was strictly dependent on external Ca^{2+}. This was shown by applying either the specific agonists AMPA and *t*-ACPD together or QA, a mixed AMPA and mGluR agonist. The AA released by veratridine was also dependent on external Ca^{2+} (Fig. 2).

Inhibition of the veratridine-induced, AMPA- plus *t*-ACPD-induced, and part of the NMDA-induced AA release by blockade of the Na^+/Ca^{2+} exchanger. Li^+ does not participate in the Na^+/Ca^{2+} exchange (34). However, Li^+ permeates sodium channels and ionotropic Glu receptor channels

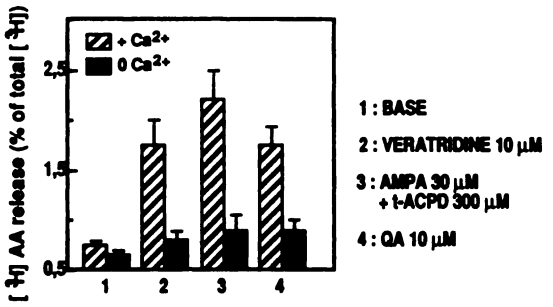

Fig. 2. Ca^{2+} dependence of [^{3}H]AA release induced by either veratridine, AMPA plus *t*-ACPD, or QA. [^{3}H]AA release was induced by the indicated agents in Krebs bicarbonate buffer; Ca^{2+} (0.75 mM) was either present or omitted during the washing and 15-min stimulation periods. Results are expressed as percentage of radioactivity released from the cells. In the histogram, each *vertical bar* represents the mean ± standard error of four independent experiments performed in triplicate.

(35). As shown in Fig. 3A, the veratridine-induced AA release was completely blocked when Na^+ was replaced by Li^+. Note that under these conditions the basal production of AA was increased (Fig. 3A). CBDMB, an amiloride analogue that inhibits the Na^+/Ca^{2+} exchanger with high potency and specificity (K_i about 1 µM) (36, 37), completely suppressed the veratridine-induced AA release, in a dose-dependent manner (EC_{50} = 562 ± 53 nM) (Fig. 3D). Replacing Na^+ with Li^+, as well as a concentration of CBDMB (30 µM) high enough to completely block the veratridine effect, also suppressed the AMPA plus *t*-ACPD stimulation of AA release (Fig. 3, B and D). The production of inositol monophosphate stimulated by *t*-ACPD was similar in the presence of Na^+ or Li^+ (data not shown). These treatments also reduced the NMDA-induced AA release, but to a lesser extent (Fig. 3, B and D).

The specificity of the CBDMB effect was strengthened by the fact that another amiloride analogue, MGCMA, which is a poor Na^+/Ca^{2+} exchanger blocker but a good Na^+/H^+ blocker (38, 39), was ineffective in blocking veratridine-, NMDA-, and AMPA- plus *t*-ACPD-induced AA release (Fig. 4A). Moreover, CBDMB (20 µM) did not significantly alter the current induced by either NMDA (50 µM) or AMPA (50 µM) (Fig. 4B). Indeed, variations induced by CBDMB in NMDA and AMPA currents were +5 ± 3% (eight experiments) and −7 ± 5% (four experiments), respectively (mean ± standard deviation). These variation values are in the range of fluctuation observed under cortical conditions in a given cell.

Absence of VSCC antagonist effect on veratridine-, NMDA-, and AMPA- plus *t*-ACPD-induced AA release. Nifedipine (10 µM), an L-type VSCC channel blocker, ω-conotoxin (10 nM), an N-type VSCC channel blocker, and cadmium (0.1 mM), a nonspecific VSCC channel blocker, had no significant effect on AA release induced by veratridine, NMDA, or AMPA plus *t*-ACPD (Table 1).

Role of PKC. Staurosporine (0.2 µM), a known inhibitor of PKC, did not inhibit AMPA- plus *t*-ACPD-, QA-, or NMDA-induced AA release. In contrast, staurosporine potentiated AMPA plus *t*-ACPD and QA responses by 72% and 130%, respectively, without affecting NMDA- or veratridine-induced AA release (Fig. 5).

Absence of effect of PTX on the release of AA induced

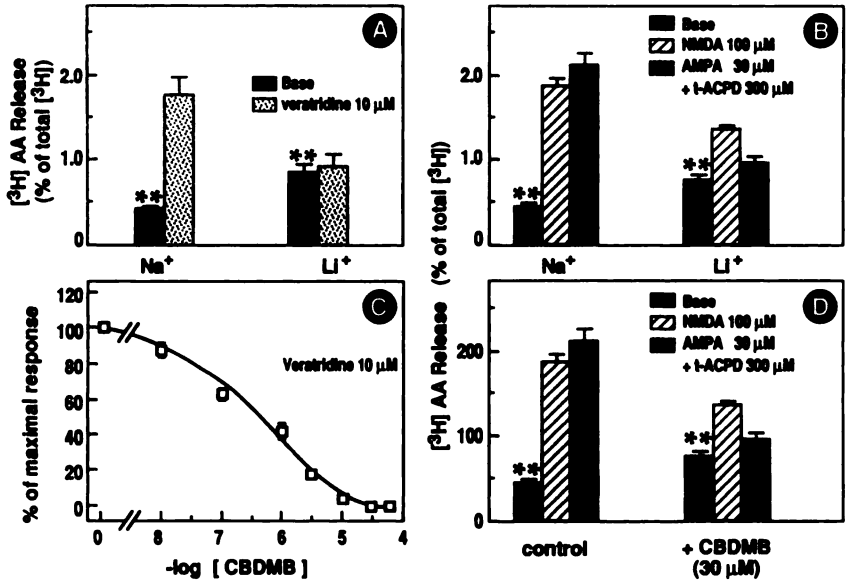

Fig. 3. Implication of the Na^+/Ca^{2+} exchanger in triggering [^{3}H]AA release. A, The percentage of radioactivity released from the cells was measured in the presence of NaCl and after substitution of NaCl with LiCl. B, NMDA- or AMPA- plus *t*-ACPD-induced AA release, expressed as percentage of radioactivity released from the cells, in the presence of NaCl and after substitution of NaCl with LiCl. C, Inhibition of veratridine-induced [^{3}H]AA release by increasing concentrations of the Na^+/Ca^{2+} exchanger inhibitor CBDMB. Neurons were preincubated for 20 min with increasing concentrations of CBDMB (0.01–60 µM) before and during the 15-min stimulation with 10 µM veratridine. Results are expressed as percentage of the maximal veratridine-induced AA release obtained in the absence of CBDMB minus basal release. Each *point* is the mean ± standard error of three separate experiments performed in duplicate. The maximum veratridine-induced [^{3}H]AA release was 2.05 ± 0.15% and the basal release was 0.55 ± 0.06% of the total radioactivity in the cells. D, Effect of CBDMB on 100 µM NMDA- or 30 µM AMPA- plus 300 µM *t*-ACPD-induced [^{3}H]AA release. Neurons were preincubated in the absence or presence of 30 µM CBDMB for 20 min before stimulation and then during the 15-min stimulation period with either NMDA or AMPA plus *t*-ACPD. Results are expressed as percentage of radioactivity released from the cells. In histograms A, B, and D, each *vertical bar* represents the mean + standard error of four independent experiments, each performed in triplicate. **, The *t* test analysis revealed a significant difference between basal AA release on one hand and AA release induced by replacing Na^+ with Li^+ or by adding CBDMB on the other hand, with $p < 0.001$.

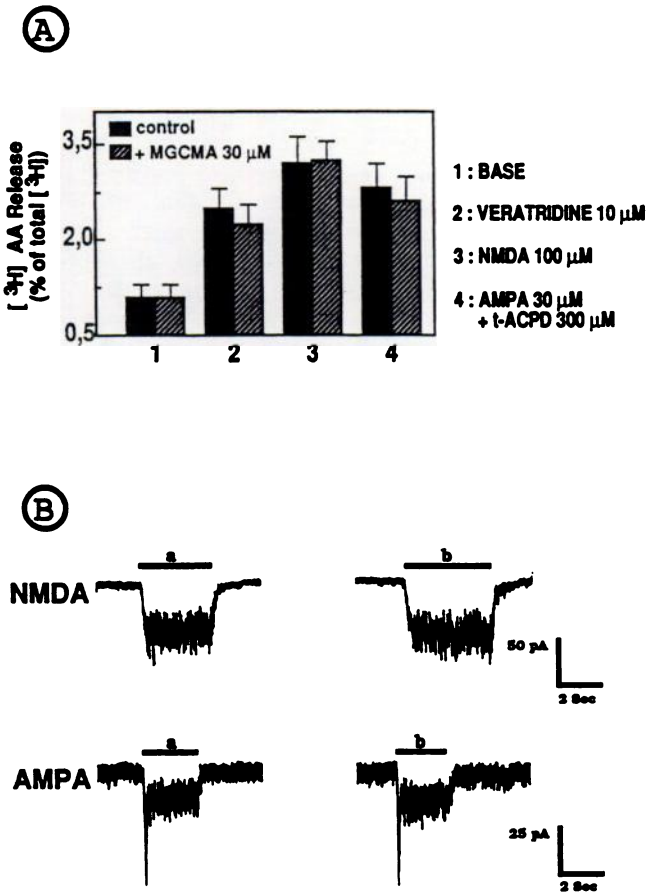

Fig. 4. Specificity of the Na^+/Ca^{2+} exchanger inhibitor CBDMB. A, Absence of effect of the Na^+/H^+ exchanger inhibitor MGCMA on [^{3}H]AA release induced by either veratridine, NMDA, or AMPA plus *t*-ACPD. Neurons were preincubated with or without 30 μM MGCMA for 20 min before stimulation and then during the 15-min stimulation period. Results are expressed as percentage of radioactivity released from the cells. In the histogram, each *vertical bar* represents the mean + standard error of four independent experiments performed in triplicate. B, Absence of effect of CBDMB on NMDA or AMPA receptors. Membrane potential was held at −60 mV and brief applications (*horizontal bars*) of either NMDA or AMPA (50 μM for both agonists) were performed in the absence (*a*) or presence (*b*) of CBDMB (30 μM). The NMDA and AMPA currents were recorded from two different cells. Similar results were obtained in 10 other cells (seven with NMDA and three with AMPA).

TABLE 1

Absence of effect of VSCC antagonists on AA release induced by different agents

Neurons were exposed to VSCC antagonists (nifedipine, ω-conotoxin, or cadmium) for 20 min before and during the 15-min stimulation period with either AMPA plus *t*-ACPD, NMDA, or veratridine. Results are expressed as percentage of stimulation over basal release. The basal release was measured in the absence of antagonists and was 0.58 ± 0.07% of the total radioactivity in the cells.

	Stimulation of AA release			
	Control	Nifedipine (10 μM)	ω-Conotoxin (10 nM)	Cd^{2+} (100 μM)
	%			
AMPA (30 μM) + *t*-ACPD (300 μM)	212 ± 21	182 ± 16	170 ± 19	230 ± 28
NMDA (100 μM)	173 ± 18	184 ± 13	169 ± 10	152 ± 17
Veratridine (10 μM)	147 ± 15	121 ± 10	130 ± 11	166 ± 13

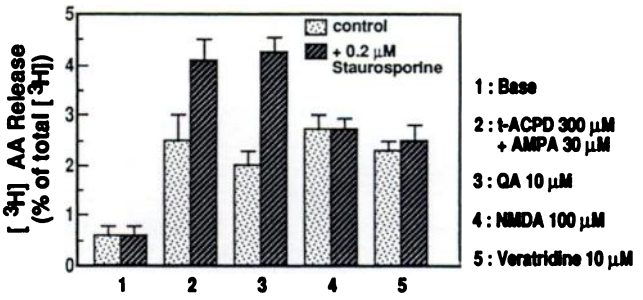

Fig. 5. Absence of PKC activation induced by the different agents evoking AA release. The effect of a PKC inhibitor, staurosporine, was tested on the [^{3}H] AA release evoked by either 30 μM AMPA plus 300 μM *t*-ACPD, 10 μM QA, 100 μM NMDA, or 10 μM veratridine. Staurosporine (0.2 μM) was added to neurons during a 20-min preincubation period and then with the stimulating agents for 15 min. Results are expressed as percentage of radioactivity released from the cells. Each *vertical bar* of the histogram is the mean + standard error of four independent experiments performed in triplicate.

by NMDA, AMPA plus *t*-ACPD, or veratridine. Pretreatment of neuronal cultures for 24 hr with 0.1 μg/ml PTX affected neither 100 μM NMDA-, 30 μM AMPA- plus 300 μM *t*-ACPD-, nor 10 μM veratridine-induced AA release (data not shown).

Discussion

One important conclusion of this study is that Ca^{2+} entry through VSCC is unable to stimulate phospholipase A_2 in striatal neurons in primary cultures. This is clearly indicated by the following results: (a) high concentrations (up to 100 mM) of K^+, which increased Ca^{2+} levels in striatal neuronal cell bodies and main dendrites up to 600 nM (data not shown), were unable to increase AA release, (b) AA release induced by veratridine was completely blocked when Na^+ was replaced by Li^+, a situation in which VSCC should be fully activated, and (c) Cd^+, nifedipine, and ω-conotoxin did not inhibit veratridine-induced AA release. These results indicate that VSCC and phospholipase A_2 are likely to be located in different neuronal compartments and that the compartment in which phospholipase A_2 is expressed should also contain NMDA receptors, as well as AMPA receptors and mGluR.

In contrast to the absence of a role for VSCC in mediating the stimulation of AA release, Ca^{2+} entry via the Na^+/Ca^{2+} exchanger was an important source of Ca^{2+} to trigger this effect after veratridine or AMPA plus *t*-ACPD stimulation. It also represented an important component of the Ca^{2+} required for NMDA receptor-induced stimulation of AA release. The Ca^{2+} entering through the NMDA receptor channels certainly constituted another source of Ca^{2+} for NMDA receptor-induced AA release. The role of the Na^+/Ca^{2+} exchanger was demonstrated by using Na^+-Li^+ substitution, a condition under which the Na^+/Ca^{2+} exchanger is not operative. We also used CBDMB, a blocker of the exchanger. The specificity of this blocker was demonstrated by the lack of effect of another amiloride analogue (MGCMA) that is a specific blocker of the Na^+/H^+ exchanger and by the absence of a direct effect of CBDMB on NMDA and AMPA receptor channel activities. Blockade of the Na^+/Ca^{2+} exchanger by Li^+ or CBDMB, which is known to increase intracellular Ca^{2+} under resting conditions (probably by suppressing the Ca^{2+} extrusion) (37), also increases basal AA release. This further indicates the importance

of Ca^{2+} accumulation in the vicinity of the Na^+/Ca^{2+} exchanger for triggering AA release.

The mechanism by which veratridine, AMPA plus *t*-ACPD, and NMDA stimulated Ca^{2+} entry via the Na^+/Ca^{2+} exchanger is likely to be the following. The Na^+/Ca^{2+} exchanger uses an electrochemical gradient for Na^+ to "pump" Ca^{2+} out and Na^+ into the cell, with a stoichiometry of 3 Na^+ to 1 Ca^{2+} (the exchanger stoichiometry is $n = 3$) (40). The depolarization and the Na^+ entry induced by veratridine, AMPA plus *t*-ACPD, or NMDA applications reduced the Na^+ electrochemical potential, and the reversal potential of the exchanger, given as $E_{NaCa} = (nE_{Na}{}^{-2}E_{Ca})/(n-2)$, became lower than the membrane potential (V_m). Under these conditions, the Na^+/Ca^{2+} exchanger "pumped" Ca^{2+} into the cell (40, 41). The $[Ca^{2+}]_i$ reached under these conditions is given by

$$[Ca^{2+}]_i = [Ca^{2+}]_0 \exp \frac{[FV_m(n-2)]}{RT} \frac{([Na^+]_i]^n}{[Na^+]_o}$$

where R is the gas constant, T is the absolute temperature, and F is Faraday's constant (42).

Why doesn't the stimulation of AMPA receptors alone induce the reversal of the exchanger and why is the associative stimulation of mGluR required? One of the reasons could be that the Na^+/Ca^{2+} exchanger, as demonstrated in squid axons and heart cells, requires a minimal $[Ca^{2+}]_i$ for activation, even when it is pumping Ca^{2+} out of the cell (43). This is certainly the consequence of the presence of a calmodulin-like sequence within the transporter (44). Therefore, by releasing Ca^{2+} from intracellular stores, mGluR probably raise $[Ca^{2+}]_i$ to "help" the activation of the Na^+/Ca^{2+} exchanger and its reversal by Na^+ entry triggered by AMPA receptor stimulation. Veratridine stimulation likely produced a situation similar to that of AMPA receptor stimulation, because mGluR potentiate veratridine-induced AA release. However, the reduction in the Na^+ electrochemical gradient was likely to be higher after veratridine, rather than AMPA, application and therefore veratridine alone was sufficient to reverse the Na^+/Ca^{2+} exchanger. Following the same hypothesis, it is possible that NMDA receptors do not require mGluR stimulation to activate the Ca^{2+} influx via the Na^+/Ca^{2+} exchanger because NMDA channels carry enough Ca^{2+} into the cell to raise Ca^{2+} to a level sufficient to occupy the exchanger intracellular Ca^{2+} sites.

Another hypothesis to explain the role of the mGluR could be the existence of a coupling between these receptors and the Na^+/Ca^{2+} exchanger. Such a coupling might exist in Purkinje cells (45), but its nature is still unknown. Our experiments exclude a role for PKC or for a guanine nucleotide-binding protein sensitive to PTX in this coupling. In view of the diversity of metabotropic receptors (6, 10–12, 46–48), several other coupling mechanism remain possible. The fact that staurosporine, a widely used PKC inhibitor, potentiated AMPA- plus mGluR-triggered AA release might reflect PKC-mediated inhibition of metabotropic receptors under physiological conditions. Indeed, we previously reported that mGluR stimulation induces PKC translocation and that PKC activation desensitizes mGluR receptors (49).

The compartment in which the reversal of the Na^+/Ca^{2+} exchanger takes place certainly has a limited volume and is possibly restricted to ionic diffusion, which can explain a rapid rise in $[Na^+]_i$. The existence of such restricted areas in small axons has been recently invoked to explain the reversal of the Na^+/Ca^{2+} exchanger that is responsible for anoxic injury in mammalian white matter (42).

This report sheds new light on the importance of the Na^+/Ca^{2+} exchanger in the mechanism of action of Glu receptors. The role of this exchanger has certainly been previously underestimated, although one report indicated that in cerebellar slices the Na^+/Ca^{2+} exchanger mediated a major part of the NMDA-induced cGMP accumulation and therefore the NMDA-induced NO synthesis.

AA has been shown to block Glu uptake. This may lead to an increase in Glu concentration in the synaptic cleft (50). AA also regulates PKC activity in neurons (51, 52) and increases NMDA receptor channel activity (53). Therefore the functional association described here between the Na^+/Ca^{2+} exchanger, phospholipase A_2, and several Glu receptors to trigger AA release is possibly of physiological relevance. The recent observation that AA, in association with mGluR activation, stimulates Glu release in synaptosomes via PKC activation certainly reinforces this possibility (54).

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