Regulation of inositol 1,4,5-trisphosphate-induced Ca^{2+} release II. Effect of cAMP-dependent protein kinase

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VOLPE, POMPEO, AND BARBARA H. ALDERSON-LANG. *Regulation of inositol 1,4,5-trisphosphate-induced Ca^{2+} release. II. Effect of cAMP-dependent protein kinase.* Am. J. Physiol. 258 (Cell Physiol. 27): C1086–C1091, 1990.—The effect of adenosine 3′,5′-cyclic monophosphate (cAMP)-dependent protein kinase (PKA) on Ca^{2+} loading, inositol 1,4,5-trisphosphate (IP_3)-induced Ca^{2+} release, and [^{3}H]IP_3 binding of canine cerebellar membrane fractions was investigated. PKA in the presence of cAMP and the catalytic subunit of PKA did not change Ca^{2+} loading yet increased the extent of IP_3-induced Ca^{2+} release by ~35%. Hill plot analysis indicated that the catalytic subunit of PKA increased the apparent Michaelis constant of IP_3-induced Ca^{2+} release twofold, from 0.3 to 0.7 μM IP_3. The protein kinase inhibitor reversed these changes. cAMP affected neither Ca^{2+} loading nor IP_3-induced Ca^{2+} release. The catalytic subunit of PKA did not appreciably affect the maximum binding and dissociation constant of [^{3}H]IP_3 binding, as judged by Scatchard analysis. Thus the catalytic subunit of PKA influences the opening of Ca^{2+} channels by IP_3 without interfering with the binding of IP_3 to its receptor sites.

adenosine 3′,5′-cyclic monophosphate; canine cerebellar membrane fractions

VARIOUS INTRACELLULAR SECOND messengers seem to interact at several levels (6, 11, 18, 21, 29) and play interconnected roles in the phenomena leading to cell activation. The phosphatidylinositol 4,5-bisphosphate (PIP_2) pathway and adenosine 3′,5′-cyclic monophosphate (cAMP) system provide key second messengers for the action of hormones, growth factors, and neurotransmitters (3, 6, 19).

Ca^{2+} can be transiently released from intracellular Ca^{2+} stores that are probably specialized organelles referred to as calciosomes (26). Inositol 1,4,5-trisphosphate (IP_3), a soluble second messenger derived from the PIP_2 pathway (3), elicits Ca^{2+} release via IP_3-gated Ca^{2+} channels yet to be identified and reconstituted. The mechanism and regulation of IP_3-induced Ca^{2+} release are actively being investigated. Given the high density of IP_3 binding sites (29), membrane vesicles derived from the cerebellum (1, 12, 13, 22, 23) have rapidly become useful models. Recently, Supattapone et al. (23) have reported that the purified rat cerebellum IP_3-receptor can be stoichiometrically phosphorylated by cAMP-dependent protein kinase (PKA). The IP_3 receptor [likely the Purkinje cell-specific cAMP-regulated membrane phosphoprotein of molecular weight ratio 260,000 (PCPP-260) of Ref. 27] is only one of the several neuronal proteins, including neurotransmitter receptors, ion channels, and synaptic vesicle-associated proteins, which have been shown to be regulated by phosphorylation (for a review see Ref. 10).

Here, we investigate the effect of PKA on IP_3-induced Ca^{2+} release and [^{3}H]IP_3 binding using canine cerebellum membrane vesicles. We confirm, in part, previous results by Supattapone et al. (23), by showing that the catalytic subunit of PKA increased the extent of IP_3-induced Ca^{2+} release without affecting [^{3}H]IP_3 binding characteristics [maximum binding (B_{max}) and dissociation constant (K_d)]. We also show, at variance with Supattapone et al. (23), that the catalytic subunit of PKA increased twofold the apparent Michaelis constant (K_m) of IP_3-induced Ca^{2+} release and that neither cAMP, PKA, nor the catalytic subunit of PKA affected Ca^{2+} loading.

The present results suggest that cAMP-dependent protein phosphorylation and/or phosphorylation of the IP_3 receptor do not affect IP_3 binding but rather the opening of the IP_3-gated Ca^{2+} channel, i.e., phosphorylation favors the transition of the IP_3-gated Ca^{2+} channel to an open state with reduced affinity for IP_3.

MATERIALS AND METHODS

Methods. Isolation of canine cerebellar crude mitochondrial pellet (P_2) and crude microsomal pellet (P_3) fractions, Ca^{2+} loading, and IP_3-induced Ca^{2+} release were carried out as described in the companion paper (25). Total Mg^{2+} and ATP concentrations in the Ca^{2+} release assay were 0.3 and 1 mM, respectively.

[^{3}H]IP_3 binding was carried out using a centrifugation assay (1, 25). Preincubation with the catalytic subunit of PKA (200 U/ml) and incubation with [^{3}H]IP_3 were carried out essentially as described by Supattapone et al. (23); 0.5 mg of membrane protein was preincubated at 37°C for 30 min in a volume of 0.425 ml, with 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), pH 7.4, 15 mM NaCl, 10 mM $MgCl_2$, 1 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EGTA), 1 mM EDTA, 50 μM ATP, in the presence or absence of 100 U of the catalytic subunit of PKA. After 30 min, 20 μl of 0.354 M EDTA, pH 12, was added to chelate all free Mg^{2+} and set the pH at 7.7, after which the samples were placed on ice. [^{3}H]IP_3 at specified concentrations (see Fig. 6) and deionized water were added to bring assay medium volume to 0.5 ml, and the

incubation was carried out at 1.6°C for 30 min. All subsequent steps were as described in the companion paper (25).

$^{45}Ca^{2+}$ loading was also measured by Millipore filtration, as described by Supattapone et al. (23), in a medium containing, in a final volume of 1 ml, 120 mM KCl, 20 mM tris(hydroxymethyl)aminomethane (Tris)-HEPES, pH 7.2, 0.3 mM $MgCl_2$, 1 mM MgATP, 0.2 mM phosphocreatine, 10 U/ml creatine kinase, 0.5 mM sodium azide, 0.5 mM EGTA, 0.4 mM $CaCl_2$ (free Ca^{2+} = 0.3 μM), tracer $^{45}CaCl_2$ (1 μCi/ml), and 0.5 mg of membrane protein. After incubation for 0–40 min at 30°C, samples were filtered through 0.45-μm Millipore filters, which were then washed with 2 × 2.5 ml of ice-cold 120 mM KCl, 20 mM Tris-HEPES, pH 7.2, and counted for radioactivity.

Materials. Materials were as reported in the companion paper (25). cAMP, PKA, protein kinase inhibitor (PKI; Sigma P5075), dioctanoyl cAMP, and the catalytic subunit of PKA were obtained from Sigma; $^{45}CaCl_2$ (sp act 0.4 mCi/ml) was from New England Nuclear.

RESULTS

Effect of cAMP. Ca^{2+} uptake and IP_3-induced Ca^{2+} release in cerebellar fractions were studied using the spectrophotometric assay described in the companion paper (25). Figure 1*A* shows that cAMP (0.1–500 μM) did not affect the Ca^{2+}-loading rate by the cerebellar P_3 fraction. The addition of cAMP (0.1–500 μM) before (Fig. 1*B*) or after (not shown) Ca^{2+} loading did not affect IP_3-induced Ca^{2+} release. Addition of the nonhydrolyzable cAMP analogue dioctanoyl cAMP (up to 50 μM) affected neither the Ca^{2+}-loading rate nor the IP_3-induced Ca^{2+} release (not shown).

Effect of the catalytic subunit of PKA. Figure 2 shows that cerebellar P_3 (control, Fig. 2*A*) was loaded with two consecutive 10-nmol $CaCl_2$ pulses (arrows) and then challenged with 10 μM IP_3 (arrowhead); ~20 nmol Ca^{2+}/mg protein was released. When the catalytic subunit of PKA (200 U/ml) was added before Ca^{2+} loading (Fig. 2*B*), the subsequent addition of 10 μM IP_3 elicited a larger Ca^{2+} release (~27.5 nmol Ca^{2+}/mg protein). Figure 3*A* shows that the catalytic subunit of PKA (1–500 U/ml) did not influence Ca^{2+}-loading rate by the cerebellar P_3 fraction. The PKA per se did not influence Ca^{2+}-loading rate either (not shown). On the other hand, the catalytic subunit of PKA increased the extent of IP_3-induced Ca^{2+} release in a concentration-dependent manner, and half-maximal stimulation of the release was attained at ~50 U/ml (Fig. 3*B*). Average stimulation of IP_3-induced Ca^{2+} release with 100 U/ml was 34.6 ± 8.4% (SD; n = 5). When the catalytic subunit of PKA (200 U/ml) was added after Ca^{2+} loading, the addition of IP_3 within 10–15 s did not elicit a larger Ca^{2+} release (not shown).

The Ca^{2+}-loading rate was unchanged, and the amount of Ca^{2+} accumulated before IP_3 addition was identical in the presence and absence of the catalytic subunit of PKA (Fig. 2 as well as Fig. 3*B*). Since Supattapone et al. (23) have mentioned that the catalytic subunit of PKA increased the extent of Ca^{2+} loading, we also measured the maximal extent of Ca^{2+} loading (i.e., Ca^{2+} capacity) using

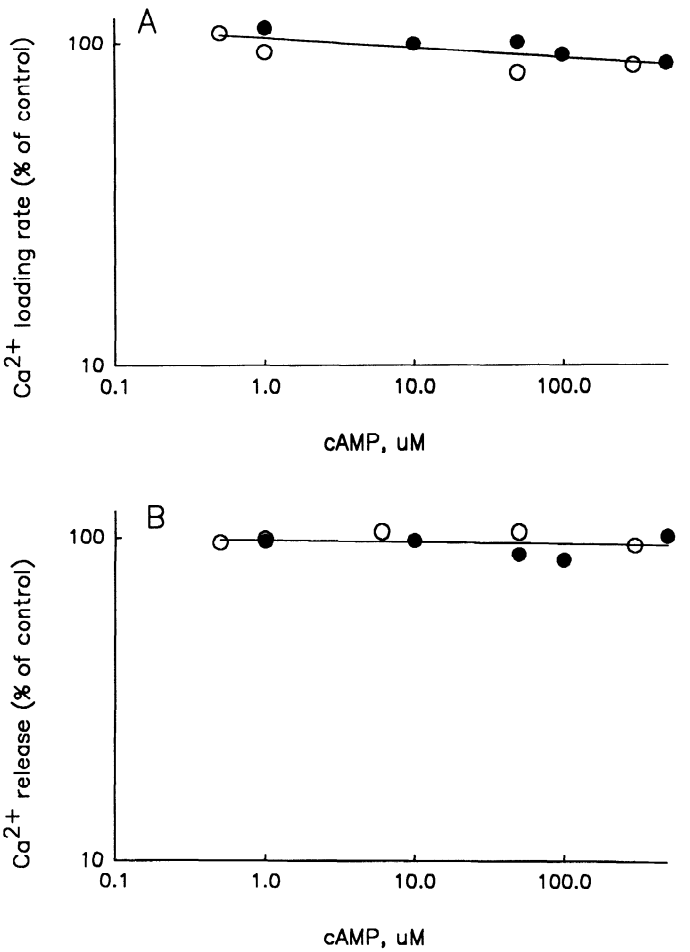

FIG. 1. Effect of cAMP on Ca^{2+} loading and inositol 1,4,5-trisphosphate (IP_3)-induced Ca^{2+} release of cerebellum crude microsomal pellet (P_3) fraction. Ca^{2+} loading and Ca^{2+} release were measured as described in MATERIALS AND METHODS using antipyrylazo III as Ca^{2+} indicator (see also Ref. 25). Total Mg^{2+} and ATP concentrations were 0.3 and 1 mM, respectively. Assay was started by adding 0.5 mg of membrane protein in presence and absence of specified concentrations of cAMP. Two consecutive 10-nmol $CaCl_2$ pulses were administered. After completion of Ca^{2+} loading, 10 μM IP_3 was added. *A*: Ca^{2+}-loading rate is expressed as %control rate (17.1 nmol $Ca^{2+} \cdot min^{-1} \cdot mg\ protein^{-1}$). Data from 2 experiments carried out on 2 different preparations are plotted (as represented by ○, ●). *B*: extent of IP_3-induced Ca^{2+} release is expressed as %control (18.9 nmol Ca^{2+}/mg protein). Data from 2 experiments carried out on 2 different preparations are plotted (as represented by ○, ●). In both panels *y*-axis values are on a log scale.

a spectrophotometric assay and an isotopic assay. With the use of the spectrophotometric assay (as in Fig. 2), P_3 fractions accumulated up to 100 nmol Ca^{2+}/mg protein, but the catalytic subunit of PKA did not increase their Ca^{2+} capacity (not shown). With the use of the isotopic assay described by Supattapone et al. (Ref. 23; see also MATERIALS AND METHODS), the catalytic subunit of PKA (200 U/ml) did not stimulate the maximal extent of Ca^{2+} loading (~20 nmol Ca^{2+}/mg protein) as shown in Fig. 4.

Effect of the catalytic subunit of PKA on the dose-response curve for IP_3-induced Ca^{2+} release. The catalytic subunit of PKA shifted the dose-response curve for IP_3-induced Ca^{2+} release to the right (Fig. 5*A*); i.e., it decreased the apparent affinity of the IP_3-sensitive Ca^{2+} channel for IP_3. Hill analysis indicated that the catalytic subunit of PKA (100 U/ml) shifted the K_m of IP_3-induced

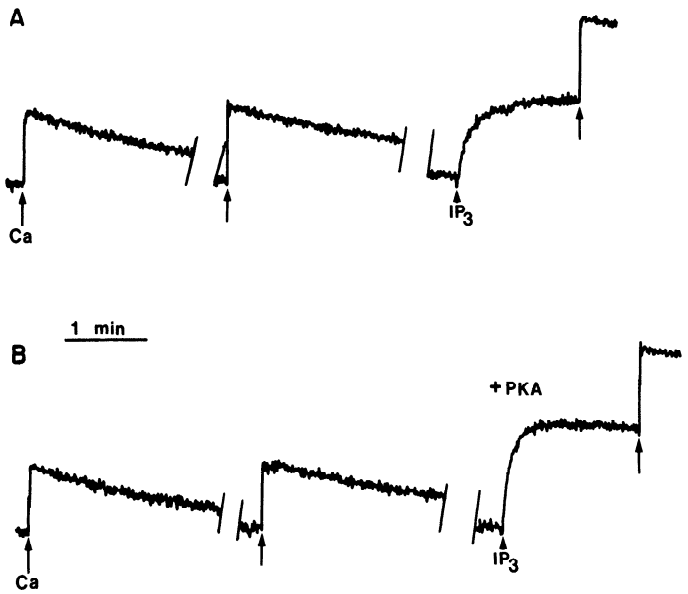

FIG. 2. Stimulation of IP_3-induced Ca^{2+} release by catalytic subunit of cAMP-dependent protein kinase (PKA). Ca^{2+} loading and release were measured as described in MATERIALS AND METHODS using antipyrylazo III as Ca^{2+} indicator (see also Ref. 25). Assay was started by adding 0.5 mg of cerebellar P_3 fraction, in absence (control; *A*) or presence of 200 U/ml catalytic subunit of PKA (*B*). Two consecutive 10-nmol $CaCl_2$ pulses were administered (arrows). After completion of Ca^{2+} loading, 10 μM IP_3 was added (arrowheads). At end of each experiment, 10 nmol $CaCl_2$ was added (arrows) to recalibrate antipyrylazo III response. Downward deflection of absorbance tracing is indicative of Ca^{2+} loading, and an upward deflection corresponds to Ca^{2+} release.

Ca^{2+} release from ~0.3 to ~0.6 μM IP_3 (Fig. 5*B*). Mean values of four experiments indicate that the catalytic subunit of PKA shifted the K_m from 0.32 ± 0.05 to 0.68 ± 0.15 (SD) μM ($P < 0.05$).

Effect of the cAMP-dependent PKA. To substantiate the observed effect of the catalytic subunit of PKA (Figs. 2 and 3), we determined the effect of PKA in the presence and absence of 0.5 mM cAMP. Table 1 shows that cAMP and PKA added separately to the Ca^{2+} loading-release medium did not modify IP_3-induced Ca^{2+} release. As expected, cAMP and PKA together mimicked the effect of the catalytic subunit and increased the extent of IP_3-induced Ca^{2+} release by ~35%. Furthermore, protein kinase inhibitor (PKI), which prevents protein kinase-dependent phosphorylation, blocked the effect of both the catalytic subunit of PKA and cAMP plus PKA.

Effect of the catalytic subunit of PKA on [^{3}H]IP_3 binding. The effect of the catalytic subunit of PKA (200 U/ml) on [^{3}H]IP_3 binding was investigated next. Before [^{3}H]IP_3 binding, cerebellar P_3 fractions were incubated in the presence or absence of the catalytic subunit of PKA, as described in MATERIALS AND METHODS, for 30 min at 37°C. Such an incubation period should allow maximal levels of phosphorylation, according to Supattopone et al. (23). The Scatchard plot of Fig. 6 indicates that the catalytic subunit of PKA did not appreciably affect the B_{max} or K_d for [^{3}H]IP_3 binding.

DISCUSSION

Both messengers generated by receptor-activated PIP_2 hydrolysis, i.e., IP_3 and diacylglycerol, might directly or

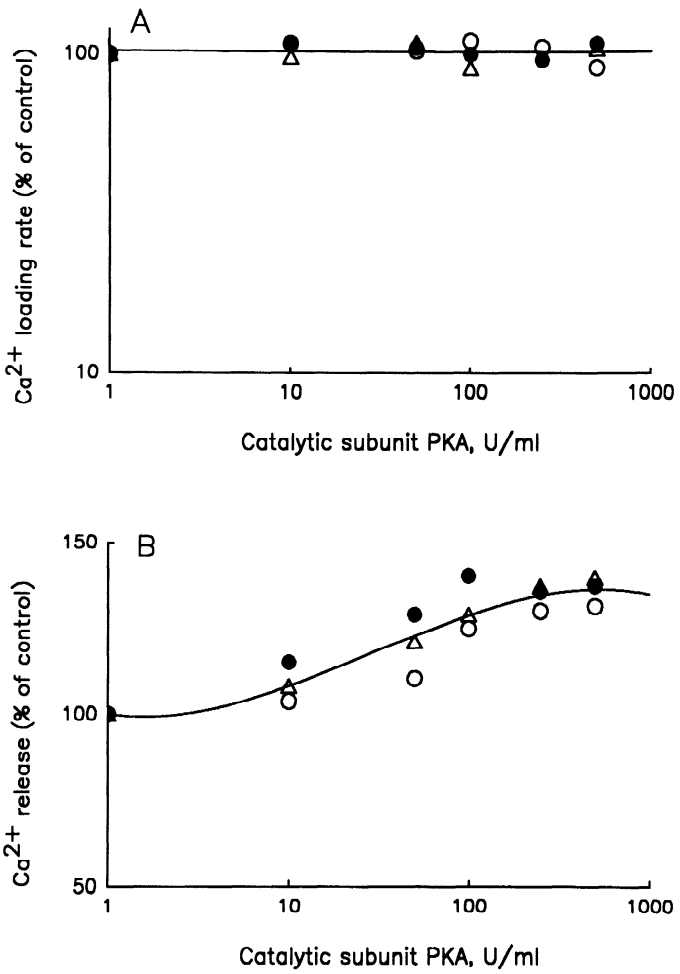

FIG. 3. Effect of catalytic subunit of PKA on Ca^{2+} loading and IP_3-induced Ca^{2+} release of cerebellar P_3 fraction. Ca^{2+} loading and release were measured as described in legend to Fig. 1, in presence or absence of catalytic subunit of PKA. *A*: Ca^{2+}-loading rate is expressed as %control rate on a log scale. Average Ca^{2+}-loading rate in presence of PKA (100 U/ml) was 94.9 ± 7.4% (±SD; $n = 5$). *B*: extent of IP_3-induced Ca^{2+} release is expressed as % of control. Data from 3 different experiments are plotted (as represented by ○, ●, and △).

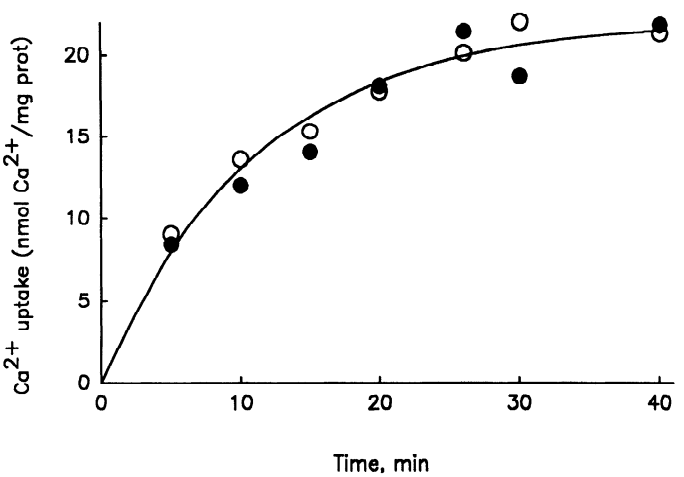

FIG. 4. Effect of catalytic subunit of PKA on Ca^{2+} capacity of cerebellar P_3 fraction. Ca^{2+} loading was carried out as described in MATERIALS AND METHODS using a Millipore filtration assay. Data points were obtained in presence (●) or absence (○) of catalytic subunit of PKA (200 U/ml). Extent of Ca^{2+} loading is smaller than in Fig. 2 because no precipitating anions are present in assay medium (13).

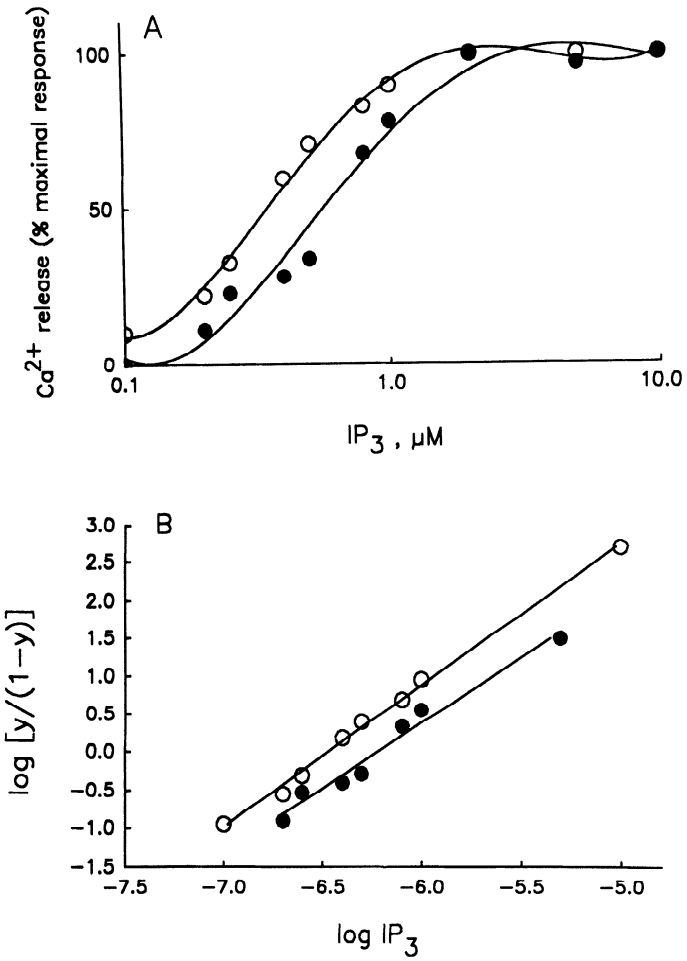

FIG. 5. Effect of catalytic subunit of PKA on dose-response curve for IP_3-induced Ca^{2+} release. IP_3-induced Ca^{2+} release was measured as described in MATERIALS AND METHODS and legend to Figs. 1–3, in presence (●) or absence (○) of PKA (100 U/ml). IP_3 concentration was varied from 0.1 to 10 μM, and total Mg^{2+} concentration was kept at 0.3 mM. *A*: typical experiment. Extent of IP_3-induced Ca^{2+} release is expressed as % of maximal response and is plotted as a function of IP_3 concentration. *B*: Hill plot of data of *A* (see also Table 1). On ordinate, *y* represents fractional release, which is defined as extent of Ca^{2+} release obtained at particular IP_3 concentration divided by maximal Ca^{2+} release obtained over all IP_3 concentrations investigated. Hill coefficients were 1.87 and 1.71 in absence and presence of PKA, respectively.

TABLE 1. *Effect of cAMP, PKA, catalytic subunit of PKA, and PKI on IP_3-induced Ca^{2+} release from cerebellar P_3 fraction*

	Extent of IP_3-Induced Ca^{2+} Release, % of control
Control	100
+cAMP	100.0±7.0* (6)
+PKA	104.5±6.6* (5)
+cAMP + PKA	132.0±7.9† (5)
+Catalytic subunit of PKA	134.6±8.4† (5)
+PKI + cAMP + PKA	97.3±5.7* (3)
+PKI + catalytic subunit of PKA	102.1±4.3* (3)

Data are means ± SD for no. of experiments shown in parentheses. PKA, cAMP-dependent protein kinase; PKI, protein kinase inhibitor; IP_3, inositol 1,4,5-trisphosphate; P_3, crude microsomal pellet. Ca^{2+} release assay was carried out as described in Ref. 25 and in legend to Fig. 2 in a medium containing 0.3 mM $MgCl_2$ and 1 mM ATP. IP_3 (10 μM) was added at completion of Ca^{2+} loading (40 nmol Ca^{2+}/mg protein). Concentrations of chemicals were as follows: 0.5 mM cAMP, 100 U/ml catalytic subunit of PKA, 100 U/ml PKA, 20 μg/ml PKI. For each set of experiments, control values for IP_3-induced Ca^{2+} release were equal to 100%. Experiments were also carried out with cerebellar crude mitochondrial pellet fractions with similar results (not shown). * P not significant in paired Student's t tests; † $P < 0.05$.

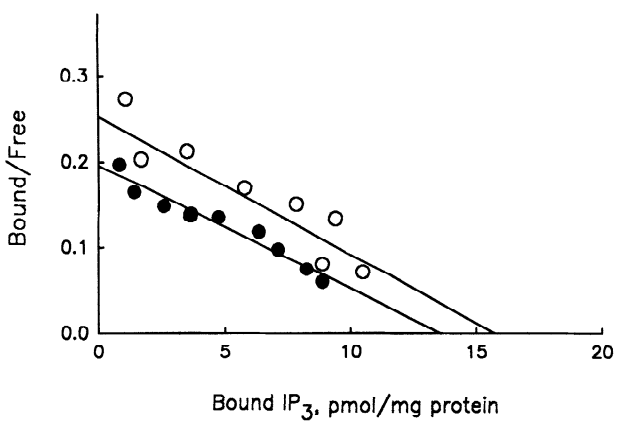

FIG. 6. Effect of catalytic subunit of PKA on [^{3}H]IP_3 binding. Experiment was carried out as described in MATERIALS AND METHODS in presence (●) or absence (○) of catalytic subunit of PKA (200 U/ml). [^{3}H]IP_3 concentration varied from 5 to 160 nM, and data are presented as Scatchard plot analysis. K_d values were 62.6 and 69.3 nM in absence or presence of catalytic subunit of PKA, respectively.

indirectly influence intracellular levels of cAMP. A relationship has been shown between stimulation of PIP_2 hydrolysis and the increase of cAMP in guinea pig brain slices, and the suggestion has been made that "generation of diacylglycerol and subsequent activation of protein kinase C are the important factors in augmented accumulations of cyclic AMP in brain slices" (11). On the other hand, the interaction between cAMP and PIP_2 turnover might occur at different levels, i.e., from the signal generation, possibly between plasma membrane receptor(s) and PIP_2 hydrolysis, to the opening of the IP_3-gated Ca^{2+} channel of intracellular Ca^{2+} stores.

IP_3 receptors (1, 23, 29) and IP_3-induced Ca^{2+} release (1, 12, 13, 22, 23) have been demonstrated in cerebellar membrane preparations. The cAMP-dependent protein kinase has been shown to be present in brain subcellular fractions (28), inside synaptosomes, and largely bound to membranes and/or cytoskeleton (4). Walaas et al. (27) have previously reported that the PCPP-260, i.e., the IP_3 receptor (23), could be phosphorylated in rat cerebellar particulate preparations by an endogenous cAMP-dependent protein kinase. Walaas et al. (27) adopted a fractionation procedure completely different from ours. The present experiments suggest that endogenous cAMP-dependent protein kinase (or a sizable portion of it) is very likely removed from the cerebellar fractions during membrane preparation and that PKA effects on IP_3-induced Ca^{2+} release probably occur through protein phosphorylation (23).

Protein phosphorylation seems to be involved for the following reasons: *1*) PKA stimulates IP_3-induced Ca^{2+} release when added before Ca^{2+} loading but not when added just before administration of IP_3, and *2*) PKI reverses PKA effects (Table 1). Although the IP_3 receptor has been reported to be phosphorylated by PKA (23) and, although two putative phosphorylation sites have

been identified in the primary sequence of the IP_3 receptor (8), we cannot rule out with certainty that other potential substrates of PKA are involved or responsible for stimulation of IP_3-induced Ca^{2+} release.

Mechanism of action of cAMP-dependent PKA on IP_3-induced Ca^{2+} release. After the initial observation of Supattapone et al. (23), we have reinvestigated, in greater detail, the effect of cAMP-dependent PKA on IP_3-induced Ca^{2+} release and [^{3}H]IP_3 binding of canine cerebellar membrane vesicles. In this paper, we show that the catalytic subunit of PKA increased the extent of IP_3-induced Ca^{2+} release without affecting Ca^{2+} preloading (Figs. 3*A* and 4). Under steady-state conditions (Fig. 3*B*), the catalytic subunit of PKA did not appear to change the intracellular-to-extracellular calcium ratio and, thus, the driving force for Ca^{2+} release. At variance with Supattapone et al. (23), we argue that the increased extent of Ca^{2+} release was not secondary to a larger Ca^{2+} preloading but was caused by a more direct effect of PKA on the IP_3-gated Ca^{2+} channel. A similar interpretation was proposed by Enouf et al. (5) to explain the stimulation by the catalytic subunit of PKA of IP_3-induced Ca^{2+} release from human platelet membrane vesicles.

The degree of stimulation of Ca^{2+} release by the catalytic subunit of PKA (~35%) can be accounted for by different, possibly interacting, mechanisms. Only a portion of IP_3 receptors may be phosphorylated in vitro, and phosphorylation may recruit additional Ca^{2+} channels. Alternatively, phosphorylation might increase the conductance and/or the open probability of the IP_3-gated Ca^{2+} channel. The ratio of Ca^{2+} released for every bound molecule of IP_3 (Figs. 2 and 6) was increased by the catalytic subunit of PKA from ~1,300:1 to 2,000:1. This observation together with the lack of a significant effect of PKA on B_{max} (Fig. 6) would be consistent with the second of the postulated mechanisms, i.e., cAMP-dependent phosphorylation seems to favor the transition of the IP_3-gated Ca^{2+} channel to an open state. However, clarification of the mechanism of action of cAMP-dependent PKA requires additional experimentation at the single-channel level, and the role of phosphatase(s) and dephosphorylation should also be investigated.

Analysis of the IP_3-induced Ca^{2+} release experiments (Figs. 2 and 5) indicates that the catalytic subunit of PKA causes a larger Ca^{2+} release via IP_3-gated Ca^{2+} channels with K_m for IP_3 increased by twofold. On the other hand, [^{3}H]IP_3 binding data (Fig. 6) indicate that the catalytic subunit of PKA does not appreciably affect the number and K_d of the [^{3}H]IP_3 binding sites. Thus the action of the catalytic subunit of PKA is distal to the binding of IP_3 to its receptor sites and is exerted on "the link between binding and Ca^{2+} release" (21) and/or on the Ca^{2+} channel (pore). The present results indicate that the IP_3 binding site and IP_3-gated Ca^{2+} channel are distinct domains of the IP_3 receptor (7).

Physiological relevance of the present data. Our data indicate that cAMP-dependent phosphorylation increases the extent and K_m of IP_3-induced Ca^{2+} release. It is tempting to speculate that, in vivo, saturating concentrations of IP_3 would always release larger amounts of Ca^{2+} and that concentrations of IP_3 well below the K_m would release less Ca^{2+} after phosphorylation (Fig. 5*A*). Thus if IP_3 and cAMP pathways are both fully activated, their combined effect should be synergistic with respect to Ca^{2+} release. On the other hand, if cAMP production is maximally activated and IP_3 production is minimal (threshold stimuli), the overall effect might be that of inhibition of IP_3-induced Ca^{2+} release.

There is evidence suggesting that, within the same neuron, receptors coupled to IP_3 production are not homogenous in their coupling mechanism (different G proteins) and in the extent of IP_3 production (2). In model neurotumor cells, forskolin, which directly activates adenylate cyclase and increases levels of cAMP, moderately potentiates IP_3-induced Ca^{2+} transients (9, but see Ref. 15). Although data on cerebellar neurons are not yet available, one can argue that, depending on the specific plasma membrane receptors involved, the interplay between the two messenger systems could produce variable cumulative effects.

The extrapolation of the present in vitro data to the in vivo situation is further complicated by *1*) additional feedback mechanisms that regulate the production of both IP_3 and cAMP (11, 18), *2*) probable decreases of cAMP levels caused by IP_3-induced Ca^{2+} release and Ca^{2+}-dependent activation of phosphodiesterase (14), and *3*) lack of specific knowledge about the distribution of IP_3-sensitive Ca^{2+} stores within neurons (18).

IP_3- and cAMP-dependent hormones act synergistically in mobilizing intracellular Ca^{2+} in liver (6). Recently, Mauger et al. (16) have reported the presence of two specific [^{3}H]IP_3 binding sites on either crude membrane fractions from rat liver or permeabilized hepatocytes and speculated that a "phosphorylation-dependent decrease of the ratio of the high to low affinity state of the (IP_3) receptor could increase the IP_3-induced Ca^{2+} release from intracellular stores." Our results cannot be directly compared with theirs, since cerebellar membrane fractions display one single class of [^{3}H]IP_3 binding sites (1, 12, 13, 23, 29) that does not appear to be influenced by phosphorylation. The IP_3 receptor has been purified only from the brain (24), and the liver IP_3 receptor might very well be different. In both tissues, however, cAMP-dependent phosphorylation seems to have a regulatory role on IP_3-induced Ca^{2+} release via a direct action on the IP_3-binding site (liver) or via an effect on the IP_3-gated Ca^{2+} channel (brain).

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