drug is not completely effective, cell life-spans may be somewhat less than we estimate. If the target cells are allowed to increase by the maximum factor observed in the five patients (that is, fivefold), we find that the derived values of c and δ are minimal estimates. Thus, for example, for data generated with $\eta = 1$, with $c = 3.00$ and $\delta = 0.500$, we find that our fitting procedure yields estimates of $c = 2.76$ and $\delta = 0.499$.

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14. Virions that are not released into the extracellular fluid are not included in this estimate. Thus, the total production in the body is even larger.
15. The solution to Eq. 3 is

$$T^*(t) = \frac{T_0^*}{c - \delta}[c \exp(-\delta t) - \delta \exp(-ct)] \quad (7)$$

If cellular RNA data were obtained, this equation could be fitted to those data, and the parameter estimates for c and δ could be verified for consistency with the viral kinetics.

16. In principle, more accurate estimates of the duration of the intracellular or eclipse phase of the viral life cycle can be obtained with a model that explicitly includes a delay from the time of infection until the time of viral release. For example, Eq. 2 can be replaced by

$$dV/dt = N\delta \int_0^\infty T^*(t - t')\omega(t')dt' - cV \quad (8)$$

where $\omega(t')$ is the probability that a cell infected at time $t - t'$ produces virus at time t. Explicit solutions to our model, with $\omega(t')$ given by a gamma distribution, will be published elsewhere (A. S. Perelson *et al.*, in preparation). Alternatively, if virally producing cells T_p rather than infected cells T^* are to be tracked, Eq. 1 can be replaced by

$$\frac{dT_p}{dt} = kT \int_0^\infty V(t - t')\omega(t')dt' - \delta T_p \quad (9)$$

Models of this type can also be solved explicitly when $\omega(t')$ is given by a gamma function. M. Nowak and A. Herz (personal communication) have solved this model for the case where $\omega(t')$ is a delta function, in which case the delay simply adds to the pharmacologic delay and Eq. 6 is regained after this combined delay. Analysis of current data by nonlinear least squares estimation has so far not allowed accurate simultaneous estimation of c, δ, and the intracellular delay. However, the qualitative effect of including the delay in the model is to increase the estimate of c, which is consistent with our claim that the values of c in Table 1 are minimal estimates. Higher values of c (hence lower values of $1/c$) will lead to increased estimates of the intracellular delay, $S - (1/c)$. Thus our estimate of the duration of the intracellular phase, as derived above and given in Table 2, is still a minimal estimate.

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Opposite Modulation of Cocaine-Seeking Behavior by D_1- and D_2-Like Dopamine Receptor Agonists

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Activation of the mesolimbic dopamine system is known to trigger relapse in animal models of cocaine-seeking behavior. We found that this "priming" effect was selectively induced by D_2-like, and not by D_1-like, dopamine receptor agonists in rats. Moreover, D_1-like receptor agonists prevented cocaine-seeking behavior induced by cocaine itself, whereas D_2-like receptor agonists enhanced this behavior. These results demonstrate an important dissociation between D_1- and D_2-like receptor processes in cocaine-seeking behavior and support further evaluation of D_1-like receptor agonists as a possible pharmacotherapy for cocaine addiction.

Relapse of cocaine use in cocaine-dependent people is often precipitated by episodes of intense drug craving even after prolonged abstinence. Cocaine craving has been described subjectively as resembling the positive or "high"-like qualities of the drug itself (*1*). In this sense, cocaine craving may differ from cravings for opiates or ethanol, which are sometimes described as a desire to alleviate the negative, withdrawal-associated symptoms of drug dependence (*1*). Both cocaine craving in humans and relapse in animal models of cocaine-seeking behavior are triggered by environmental stimuli associated with the drug experience (*2, 3*) and by low doses of cocaine itself (*3, 4*).

The priming effects of such cues in animal models of cocaine-seeking behavior can be mimicked by activation of the mesolimbic dopamine system (*5*), which is a major neural substrate of cocaine reinforcement (*6*). Dopamine acts at two general classes of dopamine receptors, termed D_1-like and D_2-like, that are distinguishable by their structural homology (*7*), opposite modulation of adenylate cyclase activity (*8*), and differential localization within the brain (*9*).

We tested the ability of full D_1- or D_2-like dopamine receptor agonists to induce relapse in an animal model of cocaine-seeking behavior. Male Sprague-Dawley rats were trained to press a lever to self-administer intravenous cocaine (*10, 11*). A daily 4-hour reinstatement procedure was followed in which rats self-administered cocaine for 2 hours, after which saline was substituted for the cocaine during the final 2 hours. During the time that saline was substituted, the rats' "nonreinforced" lever-press responses progressively diminished, a behavioral phenomenon known as extinction. After responding had diminished (*11*), the rats were given intraperitoneal priming injections of either the D_2-like selective receptor agonists 7-hydroxy-*N*,*N*-di-n-propyl-2-aminotetralin (7-OH-DPAT) (*12*) or quinpirole (*13*), or the D_1-like selective receptor agonist SKF 82958 (*14*). Although these dopamine agonists can selectively discriminate the D_1- from the D_2-like class of receptors, they do not adequately distinguish the various subtypes within each class in vivo. The priming ability of these dopamine receptor agonists was assessed by their ability to reinstate nonreinforced lever-pressing for saline infusions at the lever that previously delivered cocaine infusions (drug-paired lever) during the cocaine phase of the test session (Fig. 1).

The D_2-like agonist 7-OH-DPAT induced large dose-related increases in nonreinforced responding at the drug-paired lever as compared with very low levels of responding induced both by the drug vehicle and at an inactive lever (Figs. 1 and 2A). Quinpirole also induced selective responding at the drug-paired lever and with higher potency but with less efficacy and dose-dependency than responding induced by 7-OH-DPAT. These differences cannot be explained by the relative selectivity or affinity of the two agonists at D_2 or D_3 receptor subtypes (*12*) and therefore probably reflect different pharmacokinetic properties of the drugs. The possibility of a generalized rate-increasing effect of the D_2-like agonists is eliminated by the lack of significant responding at the inactive lever and by previous studies in which D_2-like agonists produced decreases rather than increases in responding when animals were treated during cocaine self-administration tests (*15, 16*). Thus, we conclude that the D_2-like agonists initiate neural processes that trigger relapse in an animal model of cocaine-seeking behavior.

In contrast to the D_2-like agonists, the

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D_1-like agonist SKF 82958 could not induce prominent responding at the drug-paired lever when tested over a wide range of doses, and caused only slight increases in lever-pressing (Figs. 1 and 2B). A similar difference in the priming ability of 7-OH-DPAT and SKF 82958 was observed after 1 day of withdrawal from cocaine. To test the possibility that SKF 82958 was behaviorally inactive, or even produced a generalized suppression of behavior, we measured horizontal locomotor activity (*17*) after injections of SKF 82958 over a 90-min period corresponding to the time period of the drug reinstatement test. A test dose of SKF 82958, which was without priming effects, produced similar (if not greater) locomotor activity as that produced by an effective priming dose of 7-OH-DPAT (Fig. 2C). Thus, the differential ability of the D_1- and D_2-like agonists to induce responding at the drug-paired lever was dissociated from their similar ability to stimulate horizontal locomotor activity.

We next tested whether pretreatment with D_1- or D_2-like agonists could modulate the priming effects of cocaine itself. Rats were given subcutaneous pretreatments with the D_1- or D_2-like agonists 30 min before they received priming infusions with low intravenous doses of cocaine (Fig. 3). Intravenous priming infusions of cocaine induced responding at the drug-paired lever in a dose-dependent fashion (Fig. 3 and Fig. 4, A through D). When rats were pretreated with the D_1-like agonist SKF 82958, the ability of cocaine to induce responding at the drug-paired lever was completely abolished (Figs. 3 and 4A). SKF 81297, another D_1-like agonist with a higher selectivity for D_1- over D_2-like receptors (*14*), also suppressed cocaine priming (Fig. 4B) but at higher doses than did SKF 82958, a result consistent with the lower potency of SKF 81297 in vivo (*14*). The ability of D_1-like agonists to block cocaine-induced responding at the drug-paired lever provides further evidence that these agonists are pharmacologically active at this time point despite their inability to prime by themselves. In contrast to the D_1-like agonists, pretreatment with the D_2-like agonist 7-OH-DPAT at a dose with low priming ability when given alone greatly enhanced the priming ability of low intravenous doses of cocaine (Figs. 3 and 4C). Therefore, D_1- and D_2-like receptor agonists exert opposing modulatory effects on cocaine priming in an animal model of cocaine-seeking behavior.

For comparison, we also tested the priming effects of caffeine in the reinstatement procedure. The stimulant properties of caffeine result from antagonism of central adenosine receptors (*18*). Caffeine was similar to the D_1-like agonist in that it lacked appreciable priming ability at doses that stimulate locomotor activity (Fig. 2, B and C). However, in contrast to pretreatment with the D_1-like agonist, and more similar to pretreatment with the D_2-like agonist, pretreatment with caffeine tended to enhance cocaine priming (Fig. 4D). Hence, the ability of SKF 82958 and SKF 81297 to abolish cocaine priming is due specifically to their D_1-like agonist properties and cannot be generalized to other locomotor stimulants, such as caffeine, that have weak inherent priming ability.

It is important to note that these D_1- and D_2-like receptor agonists are them-

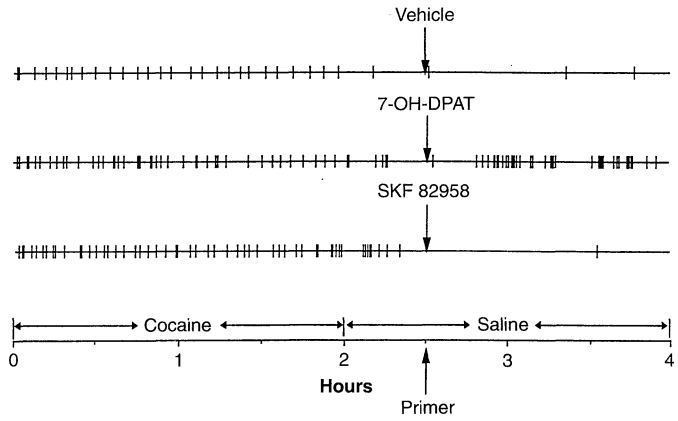

Fig. 1. Effects of intraperitoneal priming injections with vehicle (saline), the D_2-like dopamine agonist 7-OH-DPAT (3.0 mg/kg), or the D_1-like agonist SKF 82958 (1.0 mg/kg) on reinstatement of nonreinforced lever-press responding in a representative animal (see text for details). Priming injections were given after extinction from 2 hours of intravenous cocaine self-administration, when only intravenous saline injections were available. Hatchmarks denote the times of each self-infusion of cocaine in the cocaine phase and of saline in the saline phase.

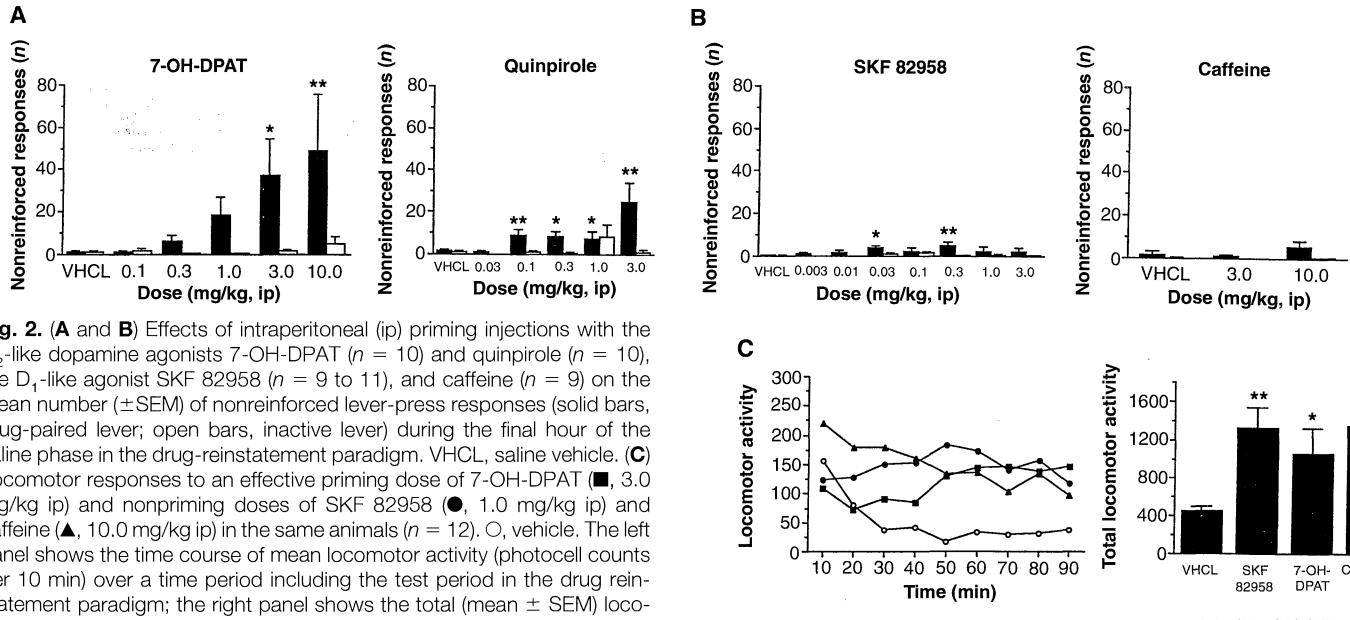

Fig. 2. (**A** and **B**) Effects of intraperitoneal (ip) priming injections with the D_2-like dopamine agonists 7-OH-DPAT (n = 10) and quinpirole (n = 10), the D_1-like agonist SKF 82958 (n = 9 to 11), and caffeine (n = 9) on the mean number (±SEM) of nonreinforced lever-press responses (solid bars, drug-paired lever; open bars, inactive lever) during the final hour of the saline phase in the drug-reinstatement paradigm. VHCL, saline vehicle. (**C**) Locomotor responses to an effective priming dose of 7-OH-DPAT (■, 3.0 mg/kg ip) and nonpriming doses of SKF 82958 (●, 1.0 mg/kg ip) and caffeine (▲, 10.0 mg/kg ip) in the same animals (n = 12). ○, vehicle. The left panel shows the time course of mean locomotor activity (photocell counts per 10 min) over a time period including the test period in the drug reinstatement paradigm; the right panel shows the total (mean ± SEM) locomotor activity (photocell counts) for the 90-min period. Asterisks in (A), (B), and (C) indicate significant difference from treatment with vehicle (VHCL) (*$P < 0.05$, **$P < 0.01$).

selves reinforcing, because each is self-administered by animals when infused intravenously (*16, 19*). Similarly, D_1- and D_2-like receptor agonists have comparable abilities to substitute for cocaine in drug discrimination tests (*20*). However, our finding that D_1- and D_2-like receptor agonists have opposite effects on cocaine priming suggests that D_1- and D_2-like receptors may mediate qualitatively different aspects of the reinforcing stimulus produced by cocaine. Thus, although D_2-like receptors may mediate the incentive to seek further cocaine reinforcement, D_1-like receptors lack this capacity and could even mediate a reduction in drive to seek further cocaine reinforcement. To study this possibility more directly, we tested the effect of pretreatment with the D_1-like agonist SKF 82958 on cocaine self-administration. Pretreatment with SKF 82958 completely suppressed the initiation of cocaine self-administration for the first half of a 2-hour test session (*21*). Moreover, there was no tolerance to this suppression during chronic intermittent treatment with SKF 82958 for 2 weeks (*21*). In contrast, pretreatment with D_2-like agonists failed to suppress the initiation of cocaine self-administration, although these drugs have been shown to decrease the rate of cocaine self-administration, presumably through an additive interaction with cocaine-reinforcement mechanisms (*15, 16*).

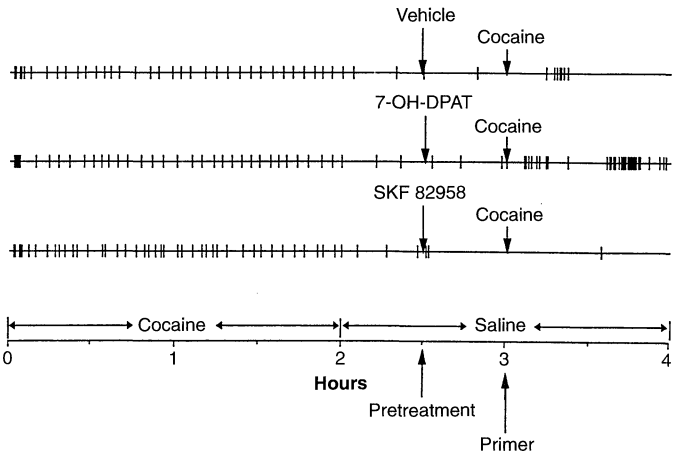

Fig. 3. Effects of subcutaneous pretreatments with the drug vehicle, the D_2-like dopamine agonist 7-OH-DPAT (0.3 mg/kg), or the D_1-like agonist SKF 82958 (1.0 mg/kg) on the ability of a low intravenous priming dose of cocaine (2.0 mg/kg) to reinstate nonreinforced lever-press responding in a representative animal (see text for details). Priming injections of cocaine were given 30 min after pretreatment with the dopamine receptor agonists during the saline phase of the reinstatement paradigm. Hatchmarks denote the times of each self-infusion of cocaine in the cocaine phase and of saline in the saline phase.

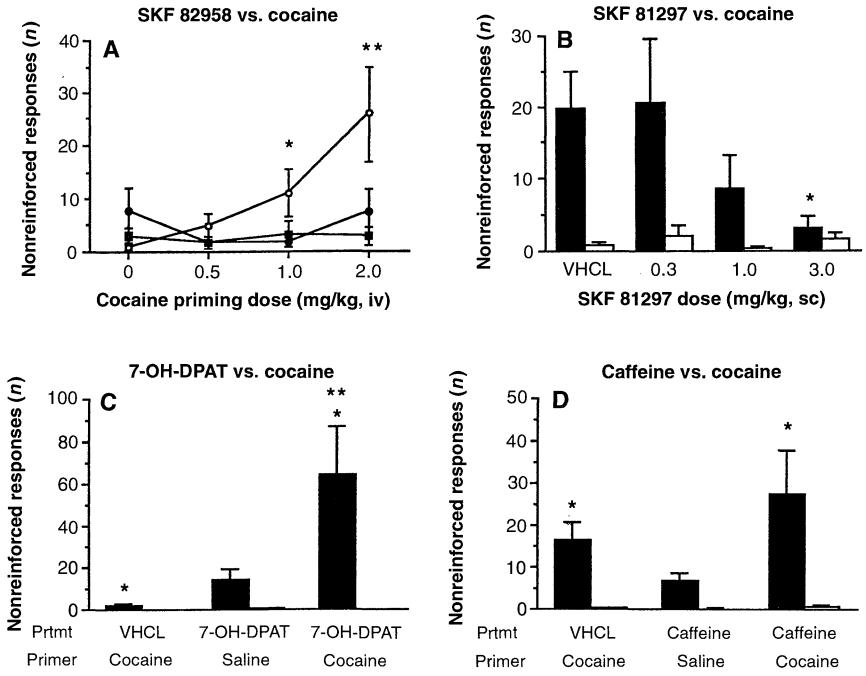

Fig. 4. (**A**) Effects of subcutaneous pretreatment with the D_1-like dopamine agonist SKF 82958 (●, 0.3 mg/kg; ■, 1.0 mg/kg) or vehicle (○) on the priming effects of intravenous (iv) doses of cocaine (n = 11). Double asterisks indicate that vehicle pretreatment differs significantly from treatment with SKF 82958 at 1.0 mg/kg ($P < 0.01$); asterisk indicates that vehicle differs significantly from SKF 82958 at 0.3 and 1.0 mg/kg ($P = 0.05$). (**B**) Effects of subcutaneous (sc) pretreatment with the highly selective D_1-like dopamine agonist SKF 81297 (n = 8 to 10) on the priming effects of iv cocaine (2.0 mg/kg). Solid bars, drug-paired lever; open bars, inactive lever. Asterisk indicates SKF 81297 treatment differs significantly from vehicle pretreatment ($P < 0.05$). (**C**) Effects of pretreatment (prtmt) with the D_2-like dopamine agonist 7-OH-DPAT (0.3 mg/kg, sc; n = 11) on the priming effects of a low-threshold dose of iv cocaine (0.5 mg/kg). Solid bars, drug-paired lever; open bars, inactive lever. Double asterisk indicates significant difference from VHCL-cocaine ($P < 0.01$); asterisk indicates significant difference from 7-OH-DPAT–saline ($P < 0.05$). (**D**) Effects of pretreatment with caffeine (10.0 mg/kg, sc; n = 9) on the priming effects of iv cocaine (2.0 mg/kg). Solid bars, drug-paired lever; open bars, inactive lever. Asterisk indicates significant difference from caffeine-saline ($P < 0.05$).

Of course the priming effects of cocaine or cocaine-associated cues could also be blocked by dopamine receptor antagonists, but these agents can exacerbate cocaine withdrawal symptoms such as anergy, anhedonia, and depression (*22*). Indeed, aversive effects have been reported for D_1- and D_2-like dopamine receptor antagonists in animals and humans (*23*), and their usefulness in treating addiction has been limited (*22*). More recently, the search for an effective pharmacotherapy for cocaine addiction has focused on dopaminergic agents with direct or indirect agonist activity at dopamine receptors as a replacement strategy (*24*). However, our results with the D_2-like agonists 7-OH-DPAT and quinpirole, together with a previous report concerning the D_2-like agonist bromocriptine (*25*), suggest that agonist activity at D_2-like receptors actually triggers relapse of cocaine-seeking behavior, at least in animals. Furthermore, in two human studies, bromocriptine failed to block subjective measures of cocaine-induced cocaine craving (*4*). Our results suggest that D_1-like receptor agonists may offer a better strategy for replacement pharmacotherapy during abstinence from cocaine. Such agents lack the inherent priming ability of D_2-like receptor agonists and block the priming effects of cocaine itself in animals. Although preliminary, these results suggest that further research is needed to determine whether D_1-like receptor agonists could prevent episodes of intense drug craving in humans and thereby prevent relapse to cocaine use.

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Coordination of Three Signaling Enzymes by AKAP79, a Mammalian Scaffold Protein

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Multivalent binding proteins, such as the yeast scaffold protein Sterile-5, coordinate the location of kinases by serving as platforms for the assembly of signaling units. Similarly, in mammalian cells the cyclic adenosine 3′,5′-monophosphate–dependent protein kinase (PKA) and phosphatase 2B [calcineurin (CaN)] are complexed by an A kinase anchoring protein, AKAP79. Deletion analysis and binding studies demonstrate that a third enzyme, protein kinase C (PKC), binds AKAP79 at a site distinct from those bound by PKA or CaN. The subcellular distributions of PKC and AKAP79 were similar in neurons. Thus, AKAP79 appears to function as a scaffold protein for three multifunctional enzymes.

Control of multiple cellular events by protein phosphorylation requires many levels of regulation in order to generate specific cellular responses. One regulatory mechanism is that kinases and phosphatases are maintained at discrete cellular locations through their interaction with targeting proteins (*1*). Enzymes may be positioned in close proximity to specific substrates, which then can be efficiently modified in response to the appropriate signals. Evidence supporting this model has shown that protein tyrosine kinases and phosphatases couple to downstream cytoplasmic enzymes through adapter proteins that contain SH2 and SH3 domains (*2*). Serine-threonine kinases and phosphatases are also maintained by scaffold proteins or anchoring proteins. In yeast, the scaffold protein Sterile-5 (STE5) provides a framework to order successive members of a yeast mitogen-activated protein kinase cascade, thereby permitting sequential activation of each enzyme in the pheromone mating response (*3*). In neurons, PKA and CaN are both localized to postsynaptic densities (PSDs) by association with AKAP79, which positions both enzymes close to key neuronal substrates (*4*). Because other neuronal signaling enzymes are present at the PSD (*5*), we investigated their potential to associate with the anchoring protein AKAP79.

PKC, a family of serine-threonine kinases, is tethered to the PSD through association with binding proteins (*6*). We used a solid-phase binding assay (overlays) with PKC as a probe (*7*) on bovine brain extracts (*8*) to detect several PKC-binding proteins, including a protein that migrated with the same mobility as a prominent RII-binding protein of 75 kD (*9*). This band corresponds to AKAP75, the bovine homolog of AKAP79 (*10*), indicating that the anchoring protein could bind both RII and PKC. Indeed, recombinant AKAP79 bound to

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