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Involvement of Calcium Channels in Depolarization-Evoked Release of Adenosine from Spinal Cord Synaptosomes

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Abstract: The potential involvement of L- and N-type voltage-sensitive calcium (Ca^{2+}) channels and a voltage-independent receptor-operated Ca^{2+} channel in the release of adenosine from dorsal spinal cord synaptosomes induced by depolarization with K^+ and capsaicin was examined. Bay K 8644 (10 nM) augmented release of adenosine in the presence of a partial depolarization with K^+ (addition of 6 mM) but not capsaicin (1 and 10 μM). This augmentation was dose dependent from 1 to 10 nM and was followed by inhibition of release from 30 to 100 nM. Nifedipine and nitrendipine inhibited the augmenting effect of Bay K 8644 in a dose-dependent manner, but neither antagonist had any effect on release of adenosine produced by K^+ (24 mM) or capsaicin (1 and 10 μM). ω -Conotoxin inhibited K^+ -evoked release of adenosine in a dose-dependent manner

but had no effect on capsaicin-evoked release. Ruthenium red blocked capsaicin-induced release of adenosine but had no effect on K^+ -evoked release. Although L-type voltage-sensitive Ca^{2+} channels can modulate release of adenosine when synaptosomes are partially depolarized with K^+ , N-type voltage-sensitive Ca^{2+} channels are primarily involved in K^+ -evoked release of adenosine. Capsaicin-evoked release of adenosine does not involve either L- or N-type Ca^{2+} channels, but is dependent on a mechanism that is sensitive to ruthenium red. **Key Words:** Adenosine release—Depolarization—Capsaicin— Ca^{2+} channels—Dihydropyridines— ω -Conotoxin—Ruthenium red. Cahill C. M. et al. Involvement of calcium channels in depolarization-evoked release of adenosine from spinal cord synaptosomes. *J. Neurochem.* 60, 886–893 (1993).

Adenosine is an important inhibitory neuromodulator in the central nervous system. In the spinal cord, adenosine plays a significant functional role in regulating nociceptive transmission (Sawynok and Sweeney, 1989). Release of adenosine contributes significantly to spinal antinociception by a number of agents including morphine and serotonin (DeLander and Hopkins, 1986, 1987; Sweeney et al., 1987, 1988), but the cellular mechanisms involved in such release are not well understood. Depolarization of synaptosomes with K^+ and capsaicin releases adenosine and nucleotide (which is converted to adenosine), respectively, from the spinal cord (Sweeney et al., 1989). In both cases, the adenosine originates from capsaicin-sensitive neurons, and release is Ca^{2+} dependent (Sweeney et al., 1987, 1988, 1989). An understanding of mechanisms underlying Ca^{2+} entry into sensory neurons may provide further insight into mechanisms involved in the spinal release of adenosine.

Ca^{2+} entry into cells occurs through either voltage-sensitive Ca^{2+} channels (VSCCs) or voltage-independent receptor-operated channels (reviewed in Bean, 1989). Three types of VSCCs (L, N, and T) have been identified in dorsal root ganglion cells (Nowicky et al., 1985a; Fox et al., 1987; Tsien et al., 1988). VSCCs are present in presynaptic nerve terminals and mediate the increase in intracellular Ca^{2+} which triggers release of neurotransmitters (Augustine et al., 1987). Several studies have examined the roles of VSCCs in mediating Ca^{2+} influx in neurotransmitter release, and VSCCs have been shown to be important for the release of dopamine (Leslie et al., 1985), acetylcholine (Suszkiw and O'Leary, 1983), and serotonin (Middlemiss and Spedding, 1985) from various central sites.

The L-type VSCC preferentially binds 1,4-dihydropyridine (DHP) analogs, such as Bay K 8644, a DHP agonist, and nifedipine, a DHP antagonist (Bean, 1989); these agents enhance and reduce, respectively, the activity of L-type Ca^{2+} channels but not T- or

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Abbreviations used: DHP, 1,4-dihydropyridine; VSCC, voltage-sensitive Ca^{2+} channel.

N-type Ca^{2+} channels (Nowycky et al., 1985a; Tsien et al., 1988). Binding studies have demonstrated the presence of DHP binding sites within the spinal cord (Gandhi and Jones, 1988), and L-type VSCCs have been implicated in modulation of the release of biogenic amines (Gandhi and Jones, 1990) and peptides (Perney et al., 1986) from the spinal cord. Other studies have indicated that the N-type Ca^{2+} channel is primarily responsible for Ca^{2+} influx into presynaptic terminals (Hirning et al., 1988; Tsien et al., 1988). ω -Conotoxin inhibits Ca^{2+} influx through the N-type VSCC and prevents release of neurotransmitters from the rat brain (Reynolds et al., 1986; Dooley et al., 1987) and spinal cord (Gandhi and Jones, 1990; Santicoli et al., 1992). Thus, both L- and N-type Ca^{2+} channels have been implicated in neurotransmitter release from spinal cord preparations.

Ca^{2+} entry into dorsal root ganglion neurons also can occur via a voltage-independent receptor-operated cation channel that is activated by capsaicin (Bevan and Szolcsányi, 1990; Holzer, 1991). Ruthenium red, an inorganic dye, has been shown to prevent the capsaicin-induced Ca^{2+} influx in sensory neurons (Wood et al., 1988; Dray et al., 1990) and to inhibit capsaicin-stimulated transmitter release from such neurons (Maggi and Meli, 1988; Amann and Maggi, 1991). Ruthenium red can thus be of considerable use in the investigation of the role of voltage-independent capsaicin-operated Ca^{2+} channels in transmitter release.

In the present study, we have examined the role of L- and N-type VSCCs in depolarization-evoked release of adenosine from spinal cord synaptosomes. This was accomplished by determining the effects of DHP analogs (Bay K 8644, nifedipine, and nitrendipine) and ω -conotoxin on K^{+} - and capsaicin-evoked release of adenosine. The actions of Bay K 8644 were examined in the presence of a range of K^{+} concentrations, as previous studies have shown a minimally or partially effective K^{+} concentration is required to elicit an effect (e.g., Gandhi and Jones, 1990). The ability of ruthenium red to modify adenosine release evoked by K^{+} and capsaicin also was evaluated.

MATERIALS AND METHODS

Preparation of spinal cord synaptosomes

Release of adenosine from spinal cord synaptosomes was studied as described previously (Sweeney et al., 1987). Briefly, male Sprague-Dawley rats weighing 250–300 g (Charles River, Quebec, Canada) were decapitated and the thoracic and lumbar spinal cords were removed quickly and hemisected into dorsal and ventral halves. Two spinal cords were used per experiment. The dorsal spinal cords were homogenized in ice-cold 0.32 M sucrose containing HEPES at pH 7.4. The synaptosomal P_2 fraction was prepared using a series of differential centrifugation steps at 4°C. Synaptosomes were then equilibrated in Krebs–Henseleit bicarbon-

ate medium at 37°C for 30 min, centrifuged at room temperature, and resuspended in Krebs–Henseleit medium at 37°C to produce a final protein concentration of 2–3 mg/ml.

Release and quantitation of adenosine

An aliquot (350 μl) of the synaptosomal suspension was added to microfuge tubes containing the drugs to be investigated. The contents of the tubes were mixed and incubated for 15 min at 37°C. Two control tubes containing only synaptosomes and medium were included in each experiment. One tube was centrifuged immediately before the 15-min incubation (time 0 min), indicating the quantity of adenosine released during the preparation of the synaptosomes. The second tube was incubated for 15 min to yield the amount of adenosine released in the absence of drugs (basal release). In all cases, release was terminated by centrifugation followed by deproteinization with ZnSO_4 and $\text{Ba}(\text{OH})_2$. The mixture was centrifuged again and the supernatant was derivatized by addition of 4.5% chloroacetaldehyde to form the etheno derivative of adenosine, which was then quantitated by HPLC with fluorescence detection. Adenosine release was expressed as picomoles per milligram of protein per 15 min. Basal adenosine values were calculated by subtracting release at 0 min from the total adenosine released in 15 min. Evoked values were calculated by subtracting the total release in the absence of drugs from total release with drugs present. When multiple doses of an agent were examined, the effects of all doses of that agent on basal release of adenosine were determined. Evoked release was then calculated by subtracting the basal value in the presence of each concentration of the drug from the treatment containing that concentration of drug.

Drugs and sources

The following compounds were used in this study: Bay K 8644 (Miles Inc., Pharmaceutical Division, Elkhart, IN, U.S.A.); nifedipine, nitrendipine, and ruthenium red (Sigma Chemical Company, St. Louis, MO, U.S.A.); and ω -conotoxin (Peninsula Labs, Belmont, CA, U.S.A.).

The DHP analogs were dissolved in 25% dimethyl sulfoxide (vol/vol), which was diluted to 0.34% (vol/vol) when incubated with synaptosomes. This concentration of dimethyl sulfoxide had no effect on basal adenosine release and was therefore considered a suitable solvent. When studying the effects of DHP analogs on adenosine release, solutions were prepared in a dark room and incubations were performed in covered water baths to minimize the degradation of these compounds during the experiment.

Statistics

Comparisons between different treatments on the same synaptosomal suspension were made using the randomized block analysis of variance with the Student–Newman–Keuls test for post hoc analysis.

RESULTS

Effects of DHP analogs on K^{+} -evoked adenosine release from rat dorsal spinal cord synaptosomes

Following the addition of increasing concentrations of K^{+} above the normal Krebs concentration (4.7 mM), there was a dose-dependent increase in the

FIG. 1. Effect of Bay K 8644 (10 nM) on the release of adenosine from spinal cord synaptosomes induced by addition of different concentrations of K⁺ above the normal concentration (4.7 mM). (●) K⁺-evoked release, (○) K⁺-evoked release in the presence of Bay K 8644 (10 nM). Values represent means ± SEM; n = 6. ***p* < 0.01 compared with K⁺-evoked adenosine release in the absence of Bay K 8644.

release of adenosine (Fig. 1). This increase was not statistically significant (at *p* < 0.05) until 12 and 24 mM K⁺ had been added. Addition of 24 mM K⁺ produced a maximal release of adenosine, as further increases in K⁺ concentrations led to no further enhancement of release (data not shown). The DHP-sensitive L-type Ca²⁺ channel agonist Bay K 8644 had no significant effect on basal adenosine release. However, in the presence of 3 and 6 mM K⁺ (total K⁺ concentration of 7.7 and 10.7 mM, respectively), a significant enhancement in adenosine release was produced by 10 nM Bay K 8644 (Fig. 1). Bay K 8644 had no effect on adenosine release in the presence of 12 or 24 mM K⁺ (total K⁺ concentrations of 16.7 and 28.7 mM, respectively). The augmentation of adenosine release produced by Bay K 8644 was dependent on concentration and exhibited a bell-shaped dose-response curve (Fig. 2). Thus, there was a concentration-dependent enhancement of release between 1 and 10 nM, but at higher concentrations (30 and 100 nM), there was a reduction in the release of adeno-

FIG. 2. Biphasic effect of Bay K 8644 on the release of adenosine from dorsal spinal cord synaptosomes induced by the addition of 6 mM K⁺ above the normal concentration (4.7 mM). Values represent means ± SEM (n = 6) of adenosine released above 6 mM K⁺ in the presence of various doses of Bay K 8644. **p* < 0.05 compared with release by 6 mM K⁺ in the absence of Bay K 8644.

sine. Bay K 8644 at concentrations lower than 1 nM and higher than 100 nM had no significant effect on release of adenosine. The DHP-sensitive L-type Ca²⁺ channel antagonists nifedipine and nitrendipine reduced the Bay K 8644-evoked enhancement of adenosine release in a dose-dependent manner (Fig. 3). These agents had no effect on adenosine released by a maximally effective concentration of K⁺ (24 mM; evoked release of 40–45 pmol/mg of protein/15 min; data not shown) or on basal release of adenosine (Fig. 3).

Effects of DHP analogs on capsaicin-evoked adenosine release

Capsaicin also evokes a dose-dependent release (1–100 μM) of adenosine from synaptosomes prepared from the dorsal spinal cord (Sweeney et al., 1989). DHP analogs were examined to determine whether L-type calcium channels were involved in capsaicin-evoked adenosine release. Neither Bay K 8644 (10 and 30 nM) nor nifedipine (100 nM) had any effect on adenosine release evoked by submaximally effective concentrations of capsaicin (1 and 10 μM; evoked release of 15–25 pmol/mg of protein/15 min; data not shown).

Effects of ω-conotoxin on release of adenosine induced by depolarization with K⁺ and capsaicin

The N-type calcium channel antagonist ω-conotoxin reduced release of adenosine evoked by addi-

FIG. 3. Effect of DHP Ca²⁺ channel antagonists, nifedipine and nitrendipine, on adenosine released by the addition of 10 nM Bay K 8644 and 6 mM K⁺. Values represent means ± SEM (n = 6) of evoked adenosine release per experiment (○). **p* < 0.05, ***p* < 0.01, ****p* < 0.001 compared with release in the absence of the dihydropyridine antagonist (●).

FIG. 4. Effects of various concentrations of ω -conotoxin (ω -ctx) on release of adenosine induced by addition of 24 mM K⁺ (A) or 10 μ M capsaicin (B) to dorsal spinal cord synaptosomes. Values depict means $\pm$ SEM of evoked adenosine release (○) ($n = 6$ for panel A; $n = 4$ for panel B). ** $p < 0.01$, *** $p < 0.001$ compared with adenosine released by the two depolarizing agents in the absence of ω -conotoxin (●).

tion of a maximally effective concentration of K⁺ (24 mM) in a concentration-dependent manner (Fig. 4A). Release of adenosine was virtually completely inhibited at 100 nM ω -conotoxin, a dose that had no effect on basal release of adenosine (see Fig. 4). In contrast to K⁺, capsaicin-evoked release of adenosine was unaffected by doses of ω -conotoxin that completely inhibited release evoked by K⁺ (Fig. 4B).

Effects of ruthenium red on release of adenosine induced by depolarization with K⁺ and capsaicin

The effects of ruthenium red on release of adenosine evoked by addition of 24 mM K⁺ and 10 μ M capsaicin were examined in a similar manner. These stimuli released a comparable quantity of adenosine from spinal cord synaptosomes. Ruthenium red reduced capsaicin-evoked release of adenosine in a dose-dependent manner (Fig. 5A). Release was completely inhibited by 10 μ M ruthenium red, a dose that had no significant effect on basal release. Ruthenium red produced similar effects on the release of adenosine evoked by 100 μ M capsaicin (evoked release of 50 pmol/mg of protein/15 min, $n = 6$) using the same dose range (data not shown). However, release of

adenosine evoked by addition of 24 mM K⁺ was unaltered by ruthenium red at doses that blocked adenosine release evoked by capsaicin (Fig. 5B). At the highest dose of ruthenium red, some reduction in the effect of K⁺ did appear to be developing, but higher doses of ruthenium red were not evaluated to test this possibility directly.

DISCUSSION

The present study demonstrates that Bay K 8644 modulates the release of adenosine from spinal cord synaptosomes slightly depolarized by addition of 3 or 6 mM K⁺, but not from synaptosomes more strongly depolarized by the addition of 24 mM K⁺. Bay K 8644 has been shown to produce a similar selective enhancement of the actions of minimally or submaximally effective concentrations of K⁺ in a number of studies. Thus, Bay K 8644 enhances Ca²⁺ uptake into rat cortical synaptosomes (White and Bradford, 1986), as well as the release of radiolabeled monoamines from spinal cord synaptosomes (Gandhi and Jones, 1990), adenosine from cerebellar neurons (Philibert and Dutton, 1989), and peptides from sensory

FIG. 5. Effects of various concentrations of ruthenium red (RR) on release of adenosine induced by addition of capsaicin (cps) (A) or K⁺ (B) to dorsal spinal cord synaptosomes. Values represent means $\pm$ SEM of evoked adenosine release (○) ($n = 6$ for panel A; $n = 4$ for panel B). * $p < 0.05$, *** $p < 0.001$ compared with adenosine released by each depolarizing agent in the absence of ruthenium red (●).

neurons (Holz et al., 1988). Partial depolarization by K^+ appears to be essential for modulation of neurotransmitter release by Bay K 8644. Experiments that vary the membrane potential with K^+ have demonstrated that Bay K 8644 interacts only with partially depolarized Ca^{2+} channels (Nowicky et al., 1985b). It thus appears that the supply of Ca^{2+} for transmitter release by L-type Ca^{2+} channels depends on presynaptic membrane potential (Huston et al., 1990).

Bay K 8644 produced a concentration-dependent enhancement of adenosine release up to 10 nM, but higher concentrations inhibited the release of adenosine. A similar bell-shaped dose-response curve for Bay K 8644 has been demonstrated for Ca^{2+} uptake during submaximal K^+ depolarization in rat cortical synaptosomes (White and Bradford, 1986), and for augmenting K^+ -evoked release of 3H -monoamines from spinal cord synaptosomes (Gandhi and Jones, 1990). It has been suggested that Bay K 8644 possesses Ca^{2+} channel antagonist actions at higher concentrations, and acts as a pure agonist only in the nanomolar dose range (White and Bradford, 1986).

Nifedipine and nitrendipine had no effect on the release of adenosine evoked by addition of a maximally effective K^+ concentration (24 mM), indicating that DHP-sensitive L-type VSCCs are not responsible for the dominant Ca^{2+} influx required for adenosine release from the spinal cord. This result is in agreement with other studies in the spinal cord where nitrendipine does not affect K^+ -stimulated 3H -monoamine release from synaptosomes (Gandhi and Jones, 1990). Studies in brain also have demonstrated that DHP antagonists have no effect on K^+ -stimulated synaptosomal Ca^{2+} entry (Nachshen and Blaustein, 1979; Daniell et al., 1983; Wei and Chiang, 1985). Both nifedipine and nitrendipine attenuated the enhanced release of adenosine produced by Bay K 8644, indicating that the modulation of Ca^{2+} entry by Bay K 8644 occurs via L-type Ca^{2+} channels. This also is consistent with findings in other studies on neurotransmitter release (Middlemiss, 1985; Herdon and Nahorski, 1989; Gandhi and Jones, 1990).

Basal release of adenosine from spinal cord synaptosomes has been shown previously to result mainly from release of nucleotide (70–80%), which subsequently is converted extrasynaptosomally to adenosine (Sweeney et al., 1987). It should be noted that the K^+ -induced release of adenosine occurs almost entirely as adenosine (80–90%; Sweeney et al., 1987). Thus, although K^+ -evoked release (40–50 pmol/mg of protein/15 min) may appear small relative to basal release (200–250 pmol/mg of protein/15 min), it represents a substantial increase in the release of adenosine per se. Throughout this series of experiments, the basal release of adenosine was not altered significantly by DHP Ca^{2+} channel agonists or antagonists. Nucleotide release is increased and adenosine release is

decreased in the absence of extracellular Ca^{2+} , whereas the opposite effects are observed in the presence of high extracellular Ca^{2+} concentrations (C. M. Cahill, T. D. White, and J. Sawynok, unpublished observations). In view of this mixed dependence of nucleotide and adenosine release on extracellular Ca^{2+} , it is perhaps not surprising that the L-type Ca^{2+} channel agents had no effect on basal adenosine release. In addition, others have suggested that Ca^{2+} entry and membrane potential of synaptosomes under resting conditions may not be adequate for activation of the L-type VSCC (White and Bradford, 1986).

In the present study, ω -conotoxin blocked K^+ -evoked release of adenosine from spinal cord synaptosomes, suggesting that N-type Ca^{2+} channels are primarily involved in the Ca^{2+} entry that initiates adenosine release during depolarization. In view of the complete nature of this blockade, it is unlikely that the recently described P-type Ca^{2+} channel (Llinas et al., 1989) is involved in this action. Our results are in agreement with other studies in the spinal cord where ω -conotoxin reduced K^+ -stimulated 3H -monoamine release from synaptosomes (Gandhi and Jones, 1990) and electrical field-stimulated release of calcitonin gene-related peptide from spinal cord slices (Santicoli et al., 1992). Studies in brain that have examined the VSCCs involved in release of [3H]serotonin (Dooley et al., 1987) and [3H]noradrenaline (Dooley et al., 1988b) have demonstrated that such release is sensitive to ω -conotoxin also but not to DHP antagonists. Although DHP analogs are specific for L-type Ca^{2+} channels, the issue of whether ω -conotoxin inhibits the N-type as well as the L-type Ca^{2+} current in neurons has been controversial using some approaches (McCleskey et al., 1987; Plummer et al., 1989). However, binding studies indicate that ω -conotoxin is specific for the N-type Ca^{2+} channel. Thus, there is no competition between the binding of ω -conotoxin and a series of drugs acting on the L-type VSCC (Cruz and Olivera, 1986), and ω -conotoxin and DHP binding sites differ in absolute number and regional distribution (Dooley et al., 1988a; Wagner et al., 1988). Although localization of specific channels to a particular region of a neuron has been proposed as a mechanism for functional specialization (Miller, 1987), L- and N-type Ca^{2+} channels coexist in both nerve terminals and cell bodies (Tsien et al., 1988). Our data are consistent with the coexistence of both channels on nerve terminals, as N-type Ca^{2+} channels appear to be involved directly in depolarization-evoked release of adenosine (addition of 24 mM K^+), whereas L-type Ca^{2+} channels can modulate K^+ -evoked release under conditions of minimal depolarization (addition of 3 and 6 mM K^+). Despite coexistence of the channels, functional specialization might occur due to the presence of specific clusters ("hot spots") containing one channel type exclusively (Fox et al., 1987; Lipscombe

et al., 1988). Accordingly, it has been suggested that Ca^{2+} influx through L-type VSCCs located at the cell body is relevant to regulation of Ca^{2+} -dependent intracellular events, whereas presynaptic localization of N-type Ca^{2+} channels is necessary for rapid generation of Ca^{2+} currents able to trigger transmitter release (Sher and Clementi, 1991).

The capsaicin-induced release of adenosine originates primarily as nucleotide, which is converted extrasynaptosomally to adenosine, rather than as adenosine per se (Sweeney et al., 1989). Capsaicin-evoked nucleotide release does not appear to involve activation of VSCCs, as neither DHP analogs nor ω -conotoxin had any effect on capsaicin-evoked release of adenosine. This result is not unexpected as a number of studies have reported that capsaicin-induced Ca^{2+} entry and neurotransmitter release is not mediated by VSCCs. Thus, nifedipine and ω -conotoxin have no effect on the capsaicin-evoked release of neuropeptides (Maggi et al., 1988, 1989) or on Ca^{2+} influx into sensory neurons (Marsh et al., 1987; Wood et al., 1988). Capsaicin-induced release of neuropeptides and Ca^{2+} influx into afferent terminals is not dependent on spinal cord membrane potential (Donnerer and Amann, 1990). The action of capsaicin on primary afferent neurons involves binding to specific sites (Maggi and Meli, 1988; Bevan and Szolcsányi, 1990) and activation of a nonselective cation inward current (Marsh et al., 1987; Wood et al., 1988), and the consequent depolarization leads to neuropeptide release (Maggi and Meli, 1988; Holzer, 1991). The Ca^{2+} influx required for capsaicin-induced peptide (Maggi and Meli, 1988) and nucleotide release (Sweeney et al., 1989) thus occurs via a voltage-independent mechanism.

Our results demonstrate that low micromolar concentrations of ruthenium red inhibit release of adenosine induced by capsaicin. Ruthenium red does not alter K^{+} -evoked release of adenosine (this study) or neuropeptides (Amann et al., 1989). Ruthenium red blocks capsaicin-evoked Ca^{2+} uptake into rat sensory neurons and inhibits capsaicin-induced neuropeptide release from sensory neurons (reviewed in Amann and Maggi, 1991). Binding studies have shown that ruthenium red does not displace binding of [^3H]-resiniferatoxin, a highly potent analog that binds competitively to the same site as capsaicin (Szallasi and Blumberg, 1990). Ruthenium red appears to act at the membrane level to interfere with the opening of capsaicin-sensitive cation channels, although the precise mechanism by which it does so is not established (Dray et al., 1990; Amann and Maggi, 1991). At higher concentrations (10 and 20 μM), some actions of ruthenium red may be due to interactions with VSCCs, as ruthenium red can displace La^{3+} binding to synaptosomes (Tapia et al., 1985), and has been shown to block K^{+} -evoked release of γ -aminop[^3H]-

butyric acid (Tapia and Meza-Ruiz, 1977). In our study, at a concentration of 10 μM , some reduction in the K^{+} -induced release of adenosine did appear to be developing, but higher concentrations were not examined to avoid this confounding action. It should be noted that although ruthenium red can block both the capsaicin- and field-stimulated release of peptides in a peripheral preparation, there is a clearly defined separation of doses required to produce these actions (Amann et al., 1990).

In conclusion, release of adenosine by depolarization with K^{+} and capsaicin involves activation of different Ca^{2+} channels. K^{+} depolarization activates N-type VSCCs, which leads to the release of adenosine. Release of adenosine by a minimally effective concentration of K^{+} is modulated by DHP analogs, indicating that L-type Ca^{2+} channels are present on the presynaptic nerve terminal. Capsaicin releases adenosine (which originates as nucleotide) by activating a ruthenium red-sensitive Ca^{2+} channel, which is not voltage dependent. The nature of the intracellular requirement for Ca^{2+} for the K^{+} -evoked release of adenosine remains to be explored, as there is no evidence that adenosine is stored in vesicles as are more classical neurotransmitters and peptides. Similarly, the nature of the nucleotide(s) released by capsaicin and the intracellular requirement for Ca^{2+} for such release also require elaboration.

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