Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 1261–1265, February 1991
Neurobiology

Multiple actions of extracellular ATP on calcium currents in cultured bovine chromaffin cells

(calcium channels/purinoceptors/modulation/GTP-binding proteins/catecholamine secretion)

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Communicated by Gerald D. Fischbach, October 29, 1990

ABSTRACT **Hormone secretion from chromaffin cells is evoked by calcium influx through voltage-dependent channels in the plasma membrane. Previous studies have shown that ATP, cosecreted with catecholamines from chromaffin granules, can modulate the secretion resulting from depolarization by nicotinic agonists. The immediate effect of ATP is to enhance secretion; more prolonged exposure to the nucleotide results in inhibition. These receptor-mediated actions of ATP involve the activation of at least two separate classes of GTP-binding protein. Results from electrophysiological experiments reported here demonstrate that the modulatory actions of ATP can, in large part, be explained by the effects of the nucleotide on inward calcium current. ATP shows a rapid enhancement and a slower, persistent inhibition of the depolarization-induced inward current.**

The importance of intracellular ATP in cellular metabolism has long been appreciated. In the past decade, it has become clear that extracellular ATP also plays a role in controlling cell function, particularly as a modulator of neurotransmission (1, 2). In many cell types, ATP is packaged in secretory vesicles, along with neurotransmitter, and is released after stimulation. It has been suggested that, in addition to its potential role as a transmitter, ATP may also regulate secretion by acting back on the nerve terminals from which it is secreted. Such a role for ATP has been best demonstrated for chromaffin cells, the catecholamine secreting cells of the adrenal medulla. These neuron-like cells have been used as a model for studying the pathways through which ATP modulates neurosecretion because dissociation of adrenal tissue provides a relatively homogeneous population of cells amenable to biochemical investigations.

The ATP released from chromaffin cells after depolarization can either enhance or inhibit catecholamine secretion, depending upon the experimental conditions (3–5). These opposing actions of ATP are mediated by distinct biochemical pathways—in particular, by different GTP-binding proteins. Pertussis toxin blocks the inhibitory actions of ATP without affecting its ability to enhance secretion. The enhancement, on the other hand, is reversed by treating chromaffin cells with cholera toxin (6, 7), which does not block the inhibition produced by ATP.

Our aim was to determine whether the inhibition and enhancement of secretion produced by extracellular ATP could be brought about through alterations in calcium influx mechanisms. We report here that ATP has multiple effects on chromaffin-cell calcium currents, which may, in large part, explain its modulatory control of secretion.

MATERIALS AND METHODS

Cell Culture. Chromaffin cells were isolated from bovine adrenal medullae according to published methods (8). Cells were plated in Eagle's minimum essential medium (GIBCO) containing 10% (vol/vol) heat-inactivated fetal calf serum (HyClone) and antibiotics at a density of $\approx 5 \times 10^5$ cells per 35-mm dish. Experiments were performed on cells between days 3 and 7 *in vitro*. Chromaffin cells could be easily distinguished from endothelial cells in the cultures by their morphology as well as by their differential uptake of neutral red.

Toxin Pretreatment. For some experiments, chromaffin-cell cultures were incubated at 37°C for 4 hr in complete medium containing either cholera toxin at 100 μg/ml (Calbiochem) or pertussis toxin at 100 ng/ml (Sigma). The toxins were stored in Locke's saline (see below) at 4°C as stock solutions of 1 mg/ml (cholera toxin) and 25 μg/ml (pertussis toxin). They were diluted to their final concentrations in medium immediately before their addition to the cultures.

Tight-seal Whole-Cell Recording. Standard giga-seal recording techniques were used (9). Patch pipettes were pulled from borosilicate glass (WPI-1BBL), fire polished, and filled with a solution containing 150 mM CsCl, 10 mM Hepes, 5 mM bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetate (BAPTA), and 5 mM MgATP, pH 7.3 (adjusted with CsOH). These pipettes had starting resistances of 5 MΩ when measured in the bath solution (Locke's) containing 154 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM glucose, 5 mM Hepes, and tetrodotoxin at 3 μg/ml, pH 7.3 (adjusted with NaOH). In some experiments, the ionic composition of the extracellular solution was altered (details given in the figure legends where relevant). Solutions of agonists were prepared daily in extracellular solution and were externally applied by pressure ejection from blunt-tipped pipettes positioned ≈20–40 μm from the cell. Experiments were performed at room temperature (21–25°C) on-line with the aid of an Atari ST computer (Instrutech, Mineola, NY). Current records were filtered at 3–10 kHz and digitized at 10–20 kHz before analysis with software distributed by Instrutech.

RESULTS

Inward Calcium Currents in Chromaffin Cells. High-threshold inward current (Fig. 1*A*) could be evoked by step depolarizations in all chromaffin cells tested ($n = 130$) under ionic conditions designed to isolate voltage-dependent calcium current (tetrodotoxin in the external and cesium in the internal solutions). The currents activated with a threshold of ≈−20 mV, maximal activation occurred near 0 mV, and the apparent reversal potential was ≈80 mV (Fig. 1*D*). Inactiva-

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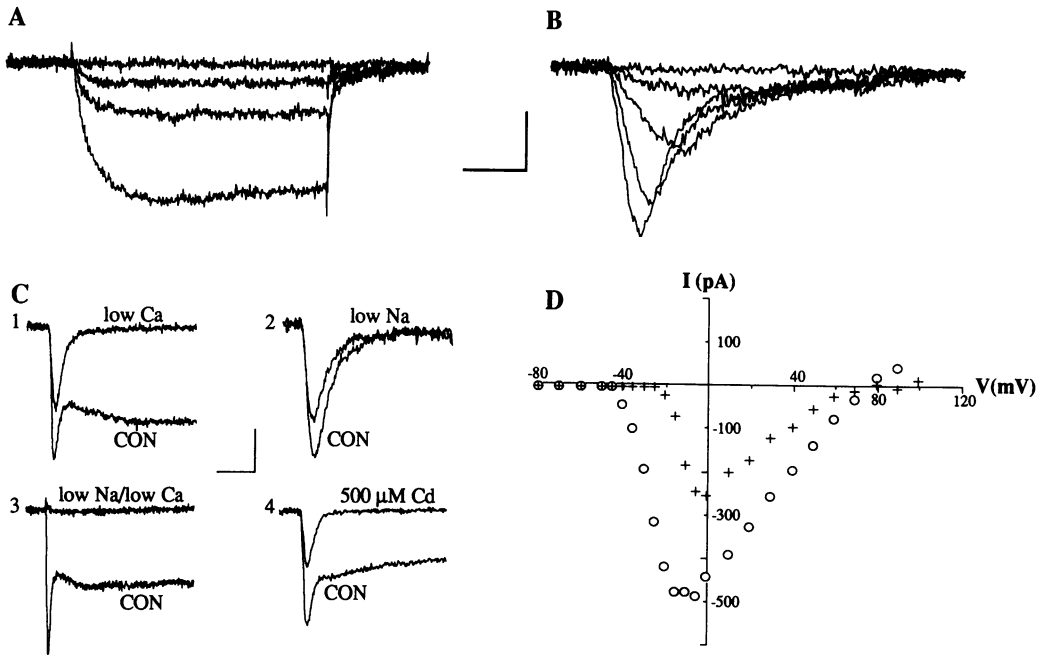

FIG. 1. Inward currents in chromaffin cells. (*A*) A family of current traces recorded from a cell that showed only a high-threshold, long-lasting component. Currents were evoked after 40-ms step depolarizations to −40, −20, −10, and 0 mV from a holding potential of −90 mV. (*B*) A family of current traces recorded from a cell that contained a substantial low-threshold, transient component. Currents were evoked after 20-ms step depolarizations to −50, −35, −30, −25, and −20 mV from a holding potential of −90 mV. Depolarizations to potentials beyond −20 mV evoked a mixed transient/long-lasting current, as shown by the traces from other cells in *C*. (Calibration: 200 pA and 10 ms in *A* and 5 ms in *B*.) (*C*) In each panel are two superimposed current records evoked by step depolarizations to −10 mV (*C1*), −20 mV (*C2*), 0 mV (*C3*), or 10 mV (*C4*) taken before (CON) and during a 10-s application of Locke's saline containing one of the following alterations: no added calcium (*C1*, low Ca), replacement of all but 10 mM sodium by sucrose (*C2*, low Na), a combination of low Ca and low Na (*C3*), and normal Locke's 500 μM cadmium chloride (*C4*). (Calibration bar for *C*: 100 pA for *C1*, *3*, and *4* and 150 pA for *C2*; 10 ms for *C1* and *3* and 5 ms for *C2* and 4.) (*D*) Current–voltage relationships for the high-threshold (+) and low-threshold (o) currents. The latter was isolated by blocking the high-threshold component with 100 μM cadmium. The high-threshold current was then determined by subtracting the transient component from total current.

tion of these high-threshold currents during step depolarizations was slow, often showing no reduction during 100-ms pulses. Steady-state inactivation of this current component was a shallow function of voltage, occurring over a 30-mV range from −50 to −20 mV. These currents are similar to the calcium currents first reported for bovine chromaffin cells by Fenwick, *et al.* (10).

In a subset of the chromaffin cells (91 of 130 cells tested), a tetrodotoxin-resistant, low-threshold component to the current was also detected (Fig. 1 *B* and *D*). Threshold for activation occurred around −45 mV, and the current reached its peak near −20 mV. Reversal of this component required depolarization to potentials positive to 75 mV. Inactivation of the low-threshold current during a step depolarization was rapid (τ = 2.5 ms at −20 mV) and very sensitive to the holding potential. Steady-state inactivation curves fit with a Boltzmann equation (data not shown) indicated that half-inactivation occurred at −73 mV, and an *e*-fold change in current was produced with a 3.6-mV change in holding potential. Not reported in previous electrophysiological studies on chromaffin cells, this current resembles low-threshold, transient calcium currents in other preparations (11–13). Further experimentation is required to fully compare this low-threshold current with these other transient calcium currents.

Both current components depend upon extracellular calcium, but the high-threshold component appears to have higher calcium-selectivity than the low-threshold component. Application of Locke's solution containing no added calcium (with an estimated free calcium concentration of 10^{-5} M) eliminated the long-lasting current but only partly reduced the transient current (Fig. 1*C1*). Fig. 1*C2* shows that the transient component was also only partially decreased by application of low-sodium Locke's solution in which the calcium concentration was normal (2.2 mM) but in which the sodium concentration was reduced from 154 mM to 10 mM (sucrose substitution). The transient current apparently can be carried by either calcium or sodium because removal of calcium from the low-sodium Locke's solution eliminated this component (Fig. 1*C3*). As has been reported for other systems in which both low- and high-threshold calcium currents coexist (11, 14), the two components were differentially sensitive to divalent cation blockade. Fig. 1*C4* illustrates that 500 μM cadmium eliminated the high-threshold but not the low-threshold component. Although a complete dose–response relationship for cadmium was not attempted, we found that lowering the cadmium concentration to 100 μM was sufficient to block 100% of the high-threshold current. This concentration was, therefore, used to construct a current–voltage relationship for the pure low-threshold current in the absence of the high-threshold component (Fig. 1*D*).

Effects of Extracellular ATP on Chromaffin-Cell Inward Currents. When ATP (magnesium salt) was externally applied to chromaffin cells at 10 or 100 μM, complex effects were observed. In most cells tested (33/34), ATP inhibited the voltage-dependent calcium current. Fig. 2*A* depicts this inhibition for a cell that exhibited only the high-threshold component of the calcium current. The effects were relatively slow in onset, reaching a peak response in 10–20 s, and occurred in the absence of any changes in holding current. The concentrations of ATP used in these studies were saturating: percentage inhibitions produced by 10 and 100 μM ATP were virtually equivalent (25% and 29%, respectively). In addition, the inhibitory effect of the nucleotide did not desensitize during ATP applications lasting several minutes. Recovery from the ATP-induced inhibition on ATP removal was usually complete, although slow, often requiring as much as 10–15 min (Fig. 2*A*). At least part of this recovery time is

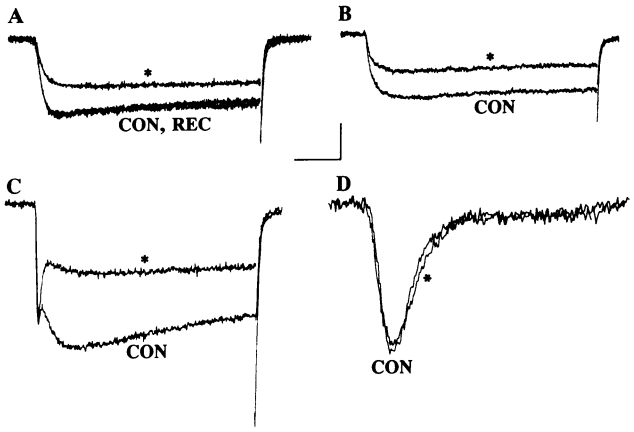

FIG. 2. Inhibition of inward currents by ATP. Superimposed current traces before (CON) and after (*) application of 100 μM ATP (*A*, *C*, and *D*) or 100 μM ADP (*B*). The recovery trace (REC) after ATP washout is also shown in *A*. Cells were held at either −80 mV (*A*, *C*, and *D*) or −60 mV (*B*). Currents were evoked from the different cells by step depolarizations to 0 mV (*A–C*) or −25 mV (*D*). (Calibration: 200 pA for *A* and *B*, 100 pA for *C*, 30 pA for *D*; 20 ms for *A* and *B*, 10 ms for *C*, 4 ms for *D*.)

a function of diffusion of agonist from the cell after application because recovery times for 10 μM ATP were significantly shorter (requiring 1.5–2 min). ATP-induced inhibition of inward current was specific for the high-threshold, long-lasting component (Fig. 2 *C* and *D*).

This inhibitory effect of ATP on the high-threshold calcium current might, at least in part, explain its action in studies of catecholamine secretion from chromaffin cells. One- to-three minute pretreatments of chromaffin cells with ATP (in the same concentration range as that used here), produce an inhibition of depolarization- or acetylcholine-induced secretion of epinephrine and norepinephrine (4). As this effect of ATP is mimicked by ADP but not by UTP (5) and blocked by pertussis toxin (6, 7), we attempted to further pursue the notion that calcium-current modulation by ATP underlies its modulation of secretion by testing the actions of ADP, UTP, and pertussis toxin in the electrophysiological experiments. ADP (100 μM) specifically inhibited the high-threshold calcium current in all cells tested (10/10) in a manner seemingly identical to that produced by ATP (Fig. 2*B*). The average reduction in current was 30%, the effects were fully reversible, and the inhibition did not desensitize. Concentrations of ADP lower than 100 μM were not tested. UTP at 100 μM was without inhibitory effect on membrane currents.

Pretreatment of chromaffin-cell cultures for 4 hr at 37°C with pertussis toxin at 100 ng/ml eliminated the ability of both ATP and ADP to produce the long-lasting inhibition of calcium current. In some toxin-treated cells, however, a fast inhibition of the current was seen during application of 100 μM ATP. This inhibition differed from that in control cells in that its recovery was rapid, occurring in <10 s. Such a rapidly reversible component was never observed in cells untreated with pertussis toxin nor in any cells tested with the lower concentration of ATP (10 μM, $n = 21$).

In a subset of the chromaffin cells tested with ATP (6 of 34), an additional response was superimposed on the inhibitory effects described above. In these cells ATP rapidly increased the inward holding current (resulting from an increase in resting membrane conductance). Additionally, after leakage subtraction (assuming linear leakage current), a concomitant potentiation of the high-threshold component of the calcium current was also apparent (Fig. 3). The time courses for these two enhancing effects of ATP were roughly similar. Precise measurement of the onset of the responses was not possible with the method of drug application used in these studies, but

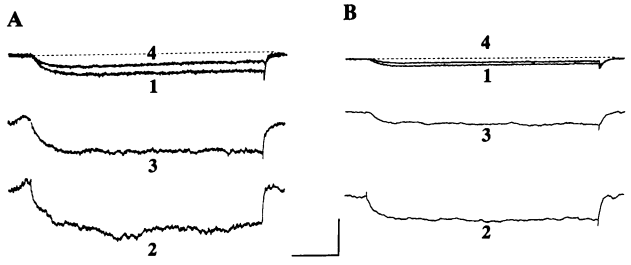

FIG. 3. Enhancement of inward currents by ATP. (*A*) Test depolarizations to 0 mV were delivered every 10 s. Trace 1 was the control, taken before 100 μM ATP application. ATP was then continuously applied to the chromaffin cell. Traces 2, 3, and 4 were taken 3, 20, and 150 s, respectively, after the start of ATP application, and all traces are plotted relative to the zero current level (dashed line) determined before ATP application. Leakage currents were determined by small depolarizing pulses near rest, scaled to the test potential assuming a linear current–voltage relationship, and subtracted from the total membrane current. All records shown have been leakage-subtracted. ATP increases the inward holding current as well as potentiating the high-threshold calcium current. Trace 2 represents the maximal enhancing effects of the nucleotide. Traces 3 and 4 illustrate the desensitization of these enhancing effects that occurs with steady application of ATP. Trace 4 also illustrates the steady inhibition of calcium current (demonstrated in Fig. 2*A*) that is uncovered after desensitization of the enhancement is complete. (*B*) Same protocol as in *A*, but the cell had been pretreated for 4 hr with cholera toxin at 100 μg/ml. Trace 1 is the control record, and traces 2–4 were taken 3, 50, and 210 s after the start of ATP application. Zero current level before ATP application is denoted by the dashed line. (Calibration: 240 pA and 20 ms for *A*, 1.7 nA and 10 ms for *B*.)

both increase in resting conductance and potentiation of voltage-dependent calcium current were maximal within ≈3 s of applying ATP. Furthermore, the magnitudes of the two effects also correlated ($r^2 = 0.94$)—the larger the increase in holding current, the larger the enhancement of high-threshold current (Fig. 4*A*). Both effects of ATP also desensitized (in the continual presence of the nucleotide) along similar time courses as shown by the raw current records in Fig. 3*A*. Further work will be required to determine whether the apparent enhancement of high-threshold current results from a specific action on the high-threshold channel or from a nonlinear ATP-gated current. In normal chromaffin cells, the process of desensitization was relatively rapid, returning to 50% of control within 20 s. As desensitization promoted the progressive return of the currents to their control levels, the steady, nondesensitizing inhibition of calcium current produced by ATP (described above) became apparent (Fig. 3, compare traces 1 and 4), suggesting that, before its complete desensitization, enhancement eclipses inhibition.

Can these effects of ATP on the membrane currents account for its stimulatory action on secretion (5)? To answer this question, we have used information from the release studies (6, 7) indicating that (*i*) ADP does not mimic the enhancing effects of ATP and (*ii*) pertussis toxin does not interfere with the ability of ATP to stimulate secretion, but (*iii*) pretreatment of chromaffin cells with cholera toxin *does* prevent the ATP-stimulated increases in catecholamine secretion. ADP (100 μM) produced neither the increase in inward holding current nor the potentiation of voltage-dependent calcium current ($n = 10$). The proportion of cells responding to 100 μM ATP with an enhancement in currents was approximately the same in control cultures (3/15) as it was in cultures treated with pertussis toxin (3/12). Pretreatment of chromaffin cell cultures for 4 hr at 37°C with cholera toxin at 100 μg/ml did not block the enhancing actions of ATP on the membrane currents, in contrast to its action on ATP-stimulated release (6, 7). The desensitization of ATP-mediated enhancement was slowed in cholera toxin-treated cells (Fig. 4*B*).

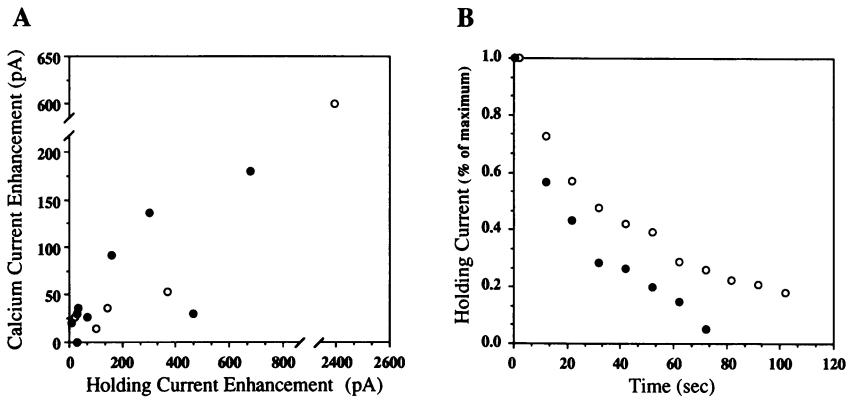

FIG. 4. Properties of ATP-induced enhancement. (*A*) Correlation between increase in holding current and potentiation of high-threshold calcium current induced by 100 μM ATP. The calcium current plotted on the ordinate was calculated as the difference between peak calcium current measured with 100 μM ATP and peak control calcium current. Each point represents measurements made from a separate cell. ●, Untreated cells; ○, cells pretreated with cholera toxin. (*B*) Time course for desensitization of the ATP-induced increase in holding current in control cell (●) and in cell pretreated with cholera toxin (○). Currents were normalized to the maximum response.

DISCUSSION

Our results demonstrate that ATP has a complex set of actions on bovine chromaffin cells. This nucleotide inhibits calcium influx, an effect that likely underlies the ATP-induced inhibition of depolarization-stimulated secretion described for these cells (3, 5). In a subpopulation of chromaffin cells, ATP increases the resting membrane conductance, which may explain the reported ability of this nucleotide to stimulate catecholamine secretion from chromaffin cells. Similar results have been reported in PC-12 cells (15). Cholera toxin pretreatment is ineffective against ATP-induced secretion and resting conductance increases. Cholera toxin does interfere with the ability of ATP to *enhance* secretion induced by acetylcholine or high potassium (6, 7). Although high-threshold calcium current appears enhanced in the presence of ATP, cholera toxin did not interfere with the effect. This result suggests that the enhancing effects of ATP on secretion are produced by an action subsequent to ion-channel activation and that cholera toxin blocks this action. Cholera toxin has been shown by others to partially inhibit secretion from these cells (16, 17).

Does a single receptor type mediate both enhancement and inhibition? A thorough pharmacological characterization of the enhancement and inhibition will have to be done to properly address this question. Certain of our results, however, suggest that separate receptors probably mediate the ATP-induced enhancement and inhibition. That nucleotide analogs exhibit specificity for either enhancement or inhibition indicates separate receptors. For example, ADP can mimic the action of ATP to inhibit but not to enhance inward current. Furthermore, the enhancement showed complete desensitization over a 2-min application of ATP, whereas the inhibition showed no desensitization over an equivalent time period. Although the desensitization may not be produced at the level of the ATP receptor, this site seems a reasonable first assumption, based on agonist-induced desensitization seen in other systems.

Is a single channel type the target for both enhancement and inhibition produced by ATP? Most evidence indicates multiple types of high-threshold, voltage-dependent calcium channel in bovine chromaffin cells. The first study of calcium currents in these cells (10) demonstrated that the deactivation kinetics of high-threshold calcium current could not be described by a single exponential, as would be expected for a single population of calcium channels. The results indicated a 10-fold difference between the fast (50 μs) and slow (500 μs) tail-current components. A recent investigation by Cena *et al.* (18) reports an adequate fit of the tail currents by a single exponential in the same cells; however, the long time constants of deactivation reported (≈3–4 ms) suggest either that a third type of calcium channel predominates in the cells under study or that the time course of the currents was limited by the recording conditions.

Biochemical and pharmacological approaches also support the notion of multiple calcium channel types in bovine chromaffin cells. Separate binding sites for two calcium channel antagonists (nitrendipine and ω-conotoxin) have been described (19). A dihydropyridine-sensitive component of ^{45}Ca uptake has been demonstrated by these investigators as well as by others (20, 21). A significant fraction (50–75%) of the ^{45}Ca uptake, however, was found to be dihydropyridine-resistant. This portion of calcium entry could be blocked by ω-conotoxin in some experiments (19) but not others (21). These results argue for distinct calcium-channel subtypes regulating calcium entry in bovine chromaffin cells.

Do multiple channel types control secretion as well? Although early reports indicated that catecholamine release was blocked completely by dihydropyridine antagonists (22), recent results are more compatible with the conclusion that, like calcium entry, secretion is controlled by multiple types of calcium channel. Boarder *et al.* (23) reported that only 70% of [^{3}H]noradrenaline release from chromaffin cells was blocked by dihydropyridine antagonists. These results compare well with those mentioned above on ^{45}Ca uptake, which indicate that a fraction of calcium entry was also insensitive to the dihydropyridines (19, 21). Taken together, the results of these ^{45}Ca uptake and catecholamine release studies suggest the likelihood that the effects of ATP to enhance and inhibit high-threshold calcium currents (reported here) and noradrenaline release (6, 7) are produced by the differential modulation of two separate channel types.

Despite the wealth of evidence implicating the high-threshold channel as the predominant mechanism for the calcium entry responsible for catecholamine secretion, a large fraction of the chromaffin cells in this study exhibited a second component of tetrodotoxin-insensitive inward current. This low-threshold current component displays some properties similar to low-threshold calcium currents in many other preparations (11–13)—in particular, its voltage-dependence of activation and sensitivity to holding potential. The channel underlying the low-threshold, transient current appears permeable to sodium and calcium because removal of both cations from Locke's solution is required for its elimination. A similar absence of high calcium selectivity has been reported for other low-threshold calcium-permeable channels as well (11), whereas voltage-dependent sodium channels are quite impermeable to calcium (24). Relative

cadmium-insensitivity is also characteristic of low-threshold as compared with high-threshold calcium currents (14). It is at present unclear whether current flowing through this channel mediates a fraction of catecholamine release. We would predict a minimal involvement of low-threshold channels in high-potassium-stimulated secretion because (*i*) the channels are completely inactivated at resting potentials of −60 mV and (*ii*) the current inactivates rapidly during depolarizing pulses. Perhaps under certain physiological conditions, however, chromaffin-cell resting potentials are negative enough to support the priming of this low-threshold component to the current. This effect, in combination with short-duration action potentials that normally trigger catecholamine secretion, might provide the conditions necessary for the low-threshold channel to play a significant role in exocytosis.

The channel(s) underlying the ATP-induced increase in inward holding current require(s) characterization, particularly with regard to calcium selectivity. It may be similar to ATP-gated channels that have been described in smooth muscle cells (25). Were such channels present in chromaffin-cell plasma membrane, their calcium permeability would allow them to play an instrumental role in the regulation of secretion and could account, at least in part, for the reported ATP-mediated increase in catecholamine secretion. It is intriguing that the presence and magnitude of the increase in holding current were correlated with that of the potentiation of voltage-dependent calcium current (recorded following subtraction of an assumed linear leak). Further characterization of the ATP-gated inward current is needed to determine whether the current–voltage relationship for the channel is linear (as reported for cesium-dialyzed sensory neurons, ref. 26) or rectifying (as recently reported for PC-12 cells, ref. 27). This important information will guide future investigations into the coordinate control of resting and voltage-dependent conductances by ATP in chromaffin cells.

The authors are indebted to Shang Zhe Xu for assistance with the chromaffin-cell cultures and for her many trips to the bus station and to Michael Goy, Timothy Turner, Daniel Cox, and Laurie Silva for critical comments on the manuscript. This work was supported by U.S. Public Health Service Grants NS16483 (K.D.), NS26606, and RR07048 (E.W.W.).

1. McAfee, D. A. & Henon, B. K. (1985) in *Adenosine & ATP in Neurotransmitter Actions in the Vertebrate Nervous System*, eds. Rogawski, M. A. & Barker, J. L. (Plenum, New York), pp. 481–502.
2. Gordon, J. L. (1986) *Biochem. J.* **233,** 309–319.
3. Kamakura, K., Sasakawa, N., Oizumi, N., Kobayashi, K. & Kuroda, Y. (1982) *Adv. Biosci.* **36,** 79–86.
4. Chern, Y. J., Kim, K. T., Slakey, L. L. & Westhead, E. W. (1988) *J. Neurochem.* **50,** 1484–1493.
5. Kim, K. T. & Westhead, E. W. (1990) *Proc. Natl. Acad. Sci. USA* **86,** 9881–9885.
6. Kim, K. T. (1989) Ph.D. thesis (Univ. of Massachusetts, Amherst).
7. Diverse-Pierluissi, M., Kopell, W. N., Kim, K. T. & Westhead, E. W. (1989) *Soc. Neurosci. Abstr.* **15,** 683.
8. Kilpatrick, D. L., Ledbetter, F. H., Carson, K. A., Kirshner, A. G., Slepatis, R. & Kirshner, N. (1980) *J. Neurochem.* **35,** 679–692.
9. Hamill, O., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981) *Pfluegers Arch.* **391,** 85–100.
10. Fenwick, E., Marty, A. & Neher, E. (1982) *J. Physiol.* **331,** 577–597.
11. Hagiwara, S., Ozawa, S. & Sand, O. (1975) *J. Gen. Physiol.* **65,** 617–644.
12. Bean, B. P. (1985) *J. Gen. Physiol.* **86,** 1–30.
13. Carbone, E. & Lux, H. D. (1987) *J. Physiol.* **386,** 547–570.
14. Fox, A. P., Nowycky, M. C. & Tsien, R. W. (1987) *J. Physiol.* **394,** 149–172.
15. Inoue, K., Nakazawa, K., Fujimori, K. & Takanaka, A. (1989) *Neurosci. Lett.* **106,** 294–298.
16. Cheek, T. R. & Burgoyne, R. D. (1987) *J. Biol. Chem.* **262,** 11663–11666.
17. Marriot, D., Adams, M. & Boarder, M. R. (1988) *J. Neurochem.* **50,** 616–623.
18. Cena, V., Stutzin, A. & Rojas, E. (1989) *J. Membr. Biol.* **112,** 255–265.
19. Ballesta, J. J., Palmero, M., Hidalgo, M. J., Gutierrez, L. M., Reig, J. A., Viniegra, S. & Garcia, A. G. (1989) *J. Neurochem.* **53,** 1050–1056.
20. Artalejo, C. R., Garcia, A. G. & Aunis, D. (1987) *J. Biol. Chem.* **262,** 915–926.
21. Rosario, L. M., Soria, B., Feuerstein, G. & Pollard, H. B. *Neuroscience* **29,** 735–747.
22. Cena, V., Nicolas, G. P., Sanchez-Garcia, P., Kirpekar, S. M. & Garica, A. G. (1983) *Neuroscience* **10,** 1455–1462.
23. Boarder, M. R., Marriott, D. & Adams, M. (1987) *Biochem. Pharmacol.* **36,** 163–167.
24. Hille, B. (1972) *J. Gen. Physiol.* **50,** 637–658.
25. Benham, C. D. & Tsien, R. W. (1987) *Nature (London)* **328,** 275–278.
26. Bean, B., Williams, C. A. & Ceelan, P. W. (1990) *J. Neurosci.* **10,** 11–19.
27. Nakazawa, K., Fugimori, K., Takanaka, A. & Inoue, K. (1990) *J. Physiol.* **428,** 257–272.