Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 3260–3264, April 1993
Physiology

T-type calcium channels in adrenal glomerulosa cells: GTP-dependent modulation by angiotensin II

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Communicated by Gerhard Giebisch, January 4, 1993

ABSTRACT **With the use of whole-cell and single-channel current recordings, we have examined in more detail the site of action of angiotensin II (AII) on multiple populations of voltage-gated calcium channels in bovine adrenal glomerulosa cells. AII (10 nM) enhances whole-cell T-type calcium channel current and increases the activity of single T-type calcium channels in cell-attached patch recordings. The AII-induced enhancement of whole-cell calcium channel currents is dependent on the presence of internal GTP and can be inhibited by the competitive AII-receptor antagonist saralasin (1 μM). These results show that AII augments the T-type calcium channel current in bovine adrenal glomerulosa cells.**

Voltage-gated calcium channels are an important site of hormone-mediated regulation of calcium influx in a variety of cells. In bovine adrenal glomerulosa cells, two distinct types of voltage-gated calcium channels have been identified (1, 2). These two distinct channel types are designated T-type and L-type. T-type voltage-gated calcium channel currents are distinguished from L-type currents by their more rapid inactivation kinetics and lower voltage threshold of activation (1–4). However, the functional importance of T-type calcium channel currents has been difficult to determine because there are very few pharmacological agents that selectively act on this class of voltage-gated calcium channel current.

Angiotensin II (AII) and, more recently, atrial natriuretic peptide have been reported to modulate T-type calcium channel current in bovine adrenal glomerulosa cells (1, 2). These peptides are the first hormones to be identified as modulators of T-type calcium channel current. However, the selectivity of hormonal action for T-type channels has been questioned. While Cohen *et al.* (1) reported AII-induced enhancement of a slowly deactivating T-type calcium channel tail current in adrenal glomerulosa cells, Hescheler *et al.* (3) reported that AII enhanced L-type calcium channel current in an adrenal carcinoma cell line (Y-1). The resolution of this issue is clearly important since AII represents only one of two hormones to date that have been reported to modulate T-type calcium channel current.

To better understand the effect of AII and to determine the messenger pathways involved in channel modulation, we have chosen to reexamine both the site and mechanism of action of AII on calcium channel current in adrenal glomerulosa cells. With the use of whole-cell and single-channel current recordings, we have found that AII modulates T-type calcium channel current in adrenal glomerulosa cells by a readily diffusible second messenger in a GTP-dependent fashion.

METHODS

Bovine Adrenal Glomerulosa Cell Isolation and Culture. Bovine adrenal glomerulosa cells were isolated as described (5). The zona glomerulosa layer was dissected from bovine adrenal cortex and placed into $CaCl_2$-free Krebs–Ringer bicarbonate (KRB) (120 mM NaCl/25 mM $NaHCO_3$/3.6 mM KCl/1.2 mM $MgSO_4$/1.2 mM NaH_2PO_4/0.1% dextrose, equilibrated with 5% CO_2/95% air). Slices were digested with collagenase [10 min at 37°C in KRB containing 0.6 mM $CaCl_2$, 0.1% bovine serum albumin (BSA), and 35 units of collagenase per mg], and cells were dispersed by mechanical agitation. Dispersed cells were filtered through 20-μm mesh (Tetko, Elmsford, NY), collected by centrifugation, and resuspended in KRB containing 1.25 mM $CaCl_2$ and 0.2% BSA, equilibrated with 95% air/5% CO_2. Cells were either used within 1–5 hr of the isolation or maintained in culture. Cells for culturing were purified on a 56% Percoll gradient, plated onto uncoated glass coverslips, and grown in 1:1 (vol/vol) Dulbecco's modified Eagle's medium/Ham's F-12 medium containing 10% (vol/vol) horse serum, 2% (vol/vol) fetal bovine serum, 100 μM ascorbate, 1.2 μM α-tocopherol, 0.05 μM Na_2SeO_3, 50 μM butylated hydroxyanisole, 5 μM metyrapone, 100 units of penicillin per ml, 100 μg of streptomycin per ml, 30 μg of gentamycin per ml, and 3 μg of amphotericin B per ml. After replacement of the serum-containing medium with serum-free medium (+ 0.2% BSA), the cells were incubated for an additional 24–30 hr before use. All single-channel recordings were performed on primary cultures of glomerulosa cells. Freshly dispersed cells were only used for whole-cell recording.

Patch-Clamp Measurements. Whole-cell calcium channel currents were recorded from cells 9–14 μm in diameter. The bath solution used for recording calcium channel currents contained 117 mM tetraethylammonium chloride, 20 mM $BaCl_2$ or $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM dextrose, 32 mM sucrose, 10 mM Hepes, and 0.2 mM tetrodotoxin (TTX) (pH 7.5; adjusted with CsOH). Bath solutions were oxygenated with 100% O_2 and maintained at room temperature. Patch pipettes (2–4 MΩ) were filled either with 108 mM CsCl/10 mM tetrabutylammonium chloride/11 mM bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetate (BAPTA)/0.9 mM $CaCl_2$/6 mM $MgCl_2$/5 mM Na_2ATP/0.04 mM GTP/10 mM Hepes, pH 7.2 (adjusted with CsOH) or with 130 mM CsCl/10 mM tetrabutylammonium chloride/11 mM EGTA/0.9 mM $CaCl_2$/2 mM $MgCl_2$/1 mM Na_2ATP/0.04 mM GTP/10 mM Hepes, pH 7.2 (adjusted with CsOH). Similar to other cell types, T-type currents were blocked by 100 μM cadmium and 50 μM nickel (2).

Membrane current was sampled at 5, 10, or 20 kHz, having been filtered with an eight-pole low-pass Bessel filter set at a cut-off frequency (−3 decibels) of 1.0, 2.5, or 5.0 kHz, respectively. For analysis, linear-leak and capacitive transient currents were subtracted digitally by appropriately scaling negative test pulses from −90 to −110 mV, which were obtained throughout the experiment. Nonlinear least-

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Abbreviations: AII, angiotensin II; GTP[γ-S], guanosine 5′-[γ-thio]triphosphate; G protein, GTP-binding regulatory protein.

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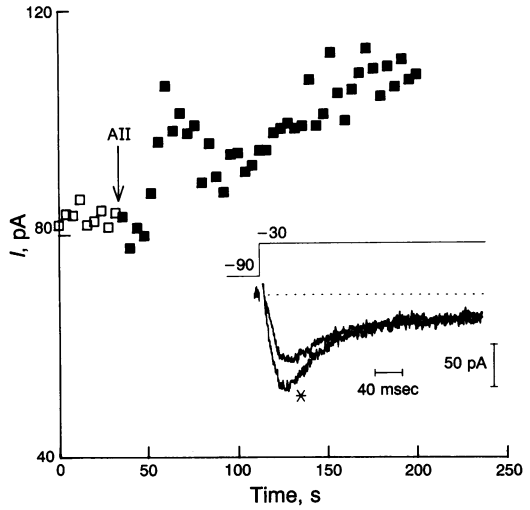

FIG. 1. AII increases low-threshold T-type calcium channel current. Absolute T-type current magnitude elicited upon step depolarization (V_t) to −30 mV from a hyperpolarized holding potential (V_h = −90 mV) is plotted versus time in seconds. Control T-type current (open squares) was stable until after the presence of AII (filled squares), when current was enhanced. (*Inset*) Low-threshold T-type current enhanced by 10 nM AII (trace marked by asterisk).

squares curve fitting was performed with the use of the Patternsearch algorithm (6) to fit activation and inactivation curves.

For cell-attached patch recordings, the recording pipette was filled with 110 mM $BaCl_2$/10 mM Hepes/200 nM TTX, pH brought to 7.4 with $Ba(OH)_2$. The bath solution contained 140 mM potassium aspartate, 10 mM dipotassium salt of EGTA, 1 mM $MgCl_2$, and 10 mM Hepes (pH 7.4 with KOH) to maintain the membrane potential at 0 mV. AII and saralasin were purchased from Sigma.

RESULTS

Time Course of AII Action on Whole-Cell T-Type Calcium Channel Currents. Fig. 1 shows the effect of 10 nM AII on whole-cell calcium channel currents evoked by weak depolarizing test potentials (V_t) to −30 mV from a holding potential (V_h) of −90 mV. With this voltage-pulse protocol, the calcium channel current is predominantly T-type since L-type current requires stronger depolarizing steps for activation (1). The magnitude of T-type current shown in Fig. 1 was stable for 5 min prior to the addition of 10 nM AII. The lack of a decline in current magnitude is consistent with the stability of whole-cell T-type calcium channel currents recorded in other preparations (1, 7). Within 30 s of adding AII to the bath (marked by arrow; filled squares), the T-type calcium channel current increased in magnitude, peaked within 30 s, declined, but then increased again in the continued presence of AII. Fig. 1 *Inset* shows T-type current records in the absence and in the presence of AII (trace marked by asterisk). The average peak increase in T-type channel current was 52.6% ± 3.83 (n = 4) determined as the percent increase in the magnitude of the slowly deactivating T-type tail current recorded upon repolarization after a 20-ms test depolarization positive to the midpoint of the tail current activation curve (V_t = +10 mV). Enhancement of T-type peak inward current or slowly deactivating tail current was observed in eight of nine whole-cell patch experiments with the time course of an increase in tail current magnitude being coincident with that of peak inward currents.

Nature of Second Messenger Generated by AII. Since GTP-binding regulatory proteins (G proteins) have been found to mediate the effects of hormones on calcium currents in a variety of other systems (8) and GTP is known to affect T-type channels in neurons (9), the dependence of AII action on the presence of GTP in the recording pipette was investigated. Fig. 2 shows sets of T-type calcium channel activation and inactivation curves from whole-cell recordings. The voltage-dependence of T-type calcium channel tail current activation recorded in control cells with 40 μM GTP in the

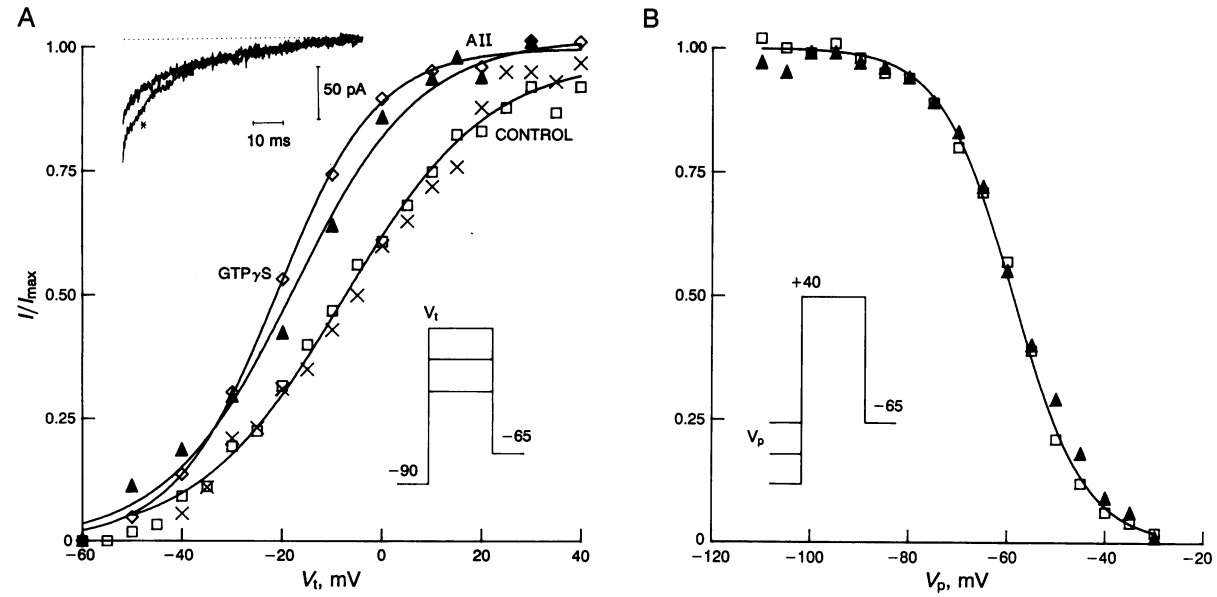

FIG. 2. (*A*) Second messenger requirement for AII action. The voltage dependence of normalized T-type calcium channel current is plotted versus test potential. In the control (open squares; 40 μM GTP in the pipette), T-type calcium channel tail current was activated with a half-maximal midpoint ($V_{1/2}$) of −7.9 mV. AII (10 nM; filled triangles) shifted the voltage dependence of T-type calcium channel activation by −10 mV ($V_{1/2}$ = −17.9 mV). In the absence of intracellular GTP, T-type calcium channel activation did not shift in response to AII [$V_{1/2}$ = −6.85 mV (points marked by ×); ΔV = +1.05]. GTP[γ-S] (40 μM; unfilled diamonds) in the pipette shifted the voltage dependence of T-type calcium channel activation without exposure to AII. (*B*) AII did not alter the voltage dependence of T-type calcium channel tail current inactivation. Control (open squares; n = 3) $V_{1/2}$ = −58.7 mV with a slope factor of 7.4; in the presence of AII (filled triangles; n = 3), $V_{1/2}$ = −57.5 with a slope factor of 7.8. Nonlinear least squares fit was to control points only.

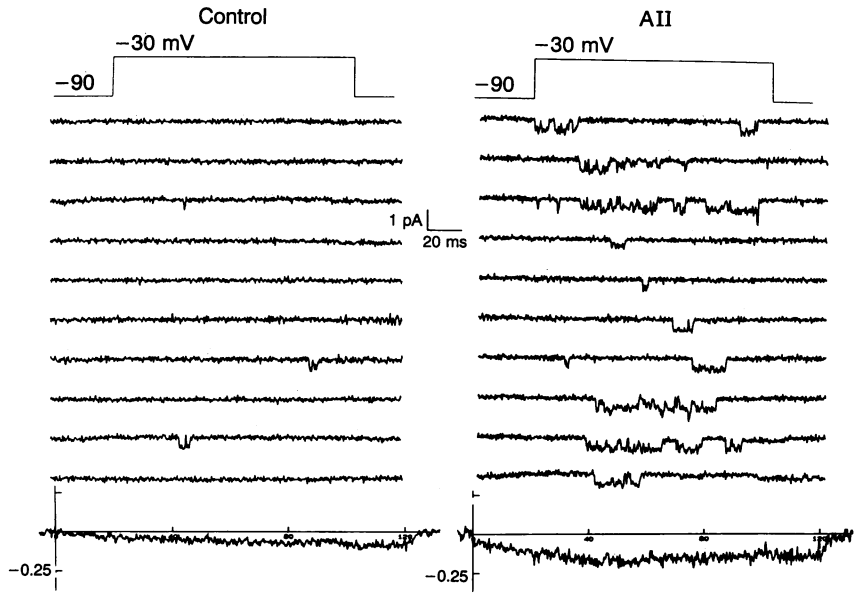

FIG. 3. AII stimulation of single T-type calcium channels. (*Left*) Control recordings (V_h = −90 mV) demonstrated openings of T-type channels (traces 3, 6, and 8) upon weak test potential depolarization (V_t = −30 mV). The bottom trace is a macroscopic single-channel current in the control averaged over 144 consecutive sweeps. (*Right*) Enhancement by 10 nM AII of T-type calcium channel opening. The averaged single-channel current (bottom trace) is markedly enhanced.

dialysate produced a midpoint of channel activation ($V_{1/2}$) of −7.9 mV ± 1.02 (n = 3; open squares in Fig. 2). Without exposure to AII, the voltage-dependence of T-type calcium channel activation with GTP in the pipette remained stable during experiments evaluated over durations of 3–21 min (ΔV = −0.36 mV ± 0.14; n = 3). However, 3 min after the addition of AII to the bath, the voltage-dependence of T-type channel activation was shifted (ΔV) in the hyperpolarizing direction by 10.0 mV ($V_{1/2}$ = −17.9 mV ± 3.23; n = 3; filled triangles in Fig. 2). Fig. 2*A Inset* shows typical slowly deactivating T-type calcium channel tail currents (V_t = −10 mV) in the control (upper trace) and in the presence of AII (lower trace marked by asterisk). This AII-induced shift in the voltage-dependence of T-type calcium channel activation upon exposure to AII did not occur when GTP was omitted from the pipette solution (n = 4; marked by ×). In three different cells, the effect of adding the nonhydrolyzable analog of GTP—namely, guanosine 5′-[γ-thio]triphosphate (GTP[γ-S]) to the pipette solution was studied. In the presence of internal GTP[γ-S], the voltage-dependence of T-type channel activation was shifted to more hyperpolarized potentials by 13 mV (ΔV = −13.4 mV; $V_{1/2}$ = −21.3 mV ± 1.0; n = 3) in the absence of AII. AII shifted the voltage dependence of activation of T-type channels without an overall increase in total current magnitude and without a change in the voltage dependence of T-type calcium channel inactivation (Fig. 2*B*). These results show that in the presence of AII, the T-type calcium channel current is activated at more negative potentials, and the action of AII is likely to involve a G protein.

Table 1. Calculation of P_o for T-type calcium channels at test potentials of −30 and −20 mV in the control and in the presence of AII

Cell	V_t, mV	P_o Control	P_o AII
1	−30	0.022	0.043
2	−30	0.016	0.050
3	−30	0.024	0.048
4A	−30	0.030	0.067
		0.023 ± 0.003*	0.052 ± 0.005*
4B	−20	0.041	0.203
5	−20	0.056	0.204
6	−20	0.048	0.067
7	−20	0.054	0.103
		0.050 ± 0.003*	0.144 ± 0.035*

*Probability of finding the channel in the open state (P_o ± SEM) for each sweep channel open time (Np) was determined by $N \cdot p = (I)/i$ (13), where i is the unitary current at test potentials of −30 mV (upper pair of values) and −20 mV (lower pair of values) and (I) is the mean value of the current.

The GTP-dependence of AII's action on T-type channels in whole-cell clamp could have occurred via either of two mechanisms. AII-activated G protein could either be acting directly on the channel [as has been observed for L-type channel by Yatani and Brown (10)] or indirectly by generating an intracellular second messenger. The cell-attached patch mode offered a means by which to address this question.

Effects of AII on Single T-Type Calcium Channels. Single-channel current recordings offer the most direct way to identify and distinguish the underlying conductances that generate the whole-cell calcium channel current. In addition, the technique of cell-attached patch recording can provide information on the molecular mechanism involved in AII receptor–channel coupling. Most importantly, the cell-attached patch mode determines whether a rapidly diffusible second messenger is generated by AII (11).

Fig. 3 shows single T-type calcium channel currents recorded in a cell-attached patch with at least two channels in the patch from a cultured adrenal glomerulosa cell (Fig. 3 *Left*). T-type currents were activated with weak test depolarization (V_t = −30 mV) from a hyperpolarized holding potential (V_h = −90 mV). With 110 mM barium as the charge carrier, the openings of T-type calcium channels are readily separable from L-type channel openings by their different conductances (12). The conductance of L-type calcium channels recorded in glomerulosa cells is 22 pS compared with 8 pS for T-type calcium channel currents. Fig. 3 shows that openings of single T-type calcium channel currents (Fig. 3

Table 2. Calculation of P_o by temporal separation of T-type calcium channels in control at a test potential of −30 mV

Cell	P_o Early	P_o Late
1	0.015	0.016
2	0.020	0.007
3	0.026	0.017
4	0.027	0.028
5	0.014	0.022
6	0.020	0.019
7	0.023	0.023
Mean	0.021 ± 0.002	0.019 ± 0.002

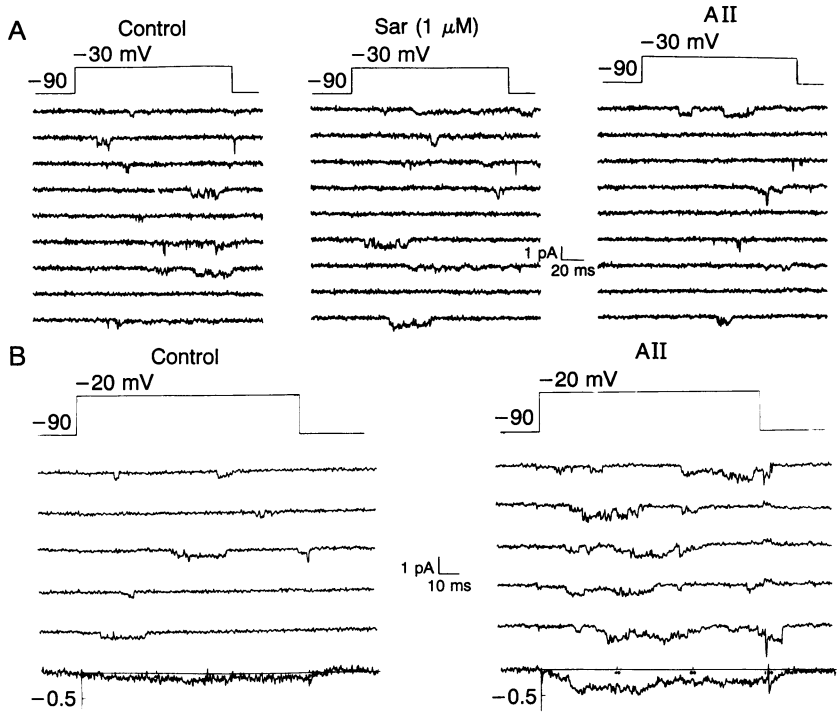

FIG. 4. Inhibition of AII-enhanced T-type calcium channel activity by saralasin (Sar) (*A Left*). T-type calcium channel opening in the control was elicited from a hyperpolarized holding potential (V_h = −90 mV) by weak test potential depolarization (V_t = −30 mV). (*A Center*) Pretreatment by 1 μM saralasin for 3 min did not alter channel activity. (*A Right*) However, saralasin pretreatment for 6 min before AII was added prevented the enhancement of T-type calcium channel activity by 10 nM AII. (*B*) AII-enhanced T-type calcium channels elicited by stronger test potential depolarization. T-type calcium channels that opened frequently upon strong test potential depolarization (V_t = −20 mV) (*Left*) were enhanced by 10 nM AII (*Right*). The last sweep of each panel is an averaged current of consecutive sweeps elicited by test depolarization to −20 mV. Because of difficulty subtracting linear-leak transient currents from traces with high P_o, small outward artifacts appear near the end of the test depolarization in some sweeps.

Left) were markedly enhanced 200 s after adding 10 nM AII to the bathing solution (Fig. 3 *Right*). The average activity of single T-type calcium channel currents was constructed from the average of 144 consecutive sweeps (0.2 Hz) and shown below each set of sweeps. The average currents demonstrate that 10 nM AII enhanced T-type current more than 2-fold compared with the control.

Channel open probability for each sweep (P_o) at −30 mV in this multichannel patch (Fig. 3) was 0.030 in the control and 0.067 in the presence of 10 nM AII (evaluated from 163 consecutive sweeps obtained both in the control and in the presence of AII). In seven separate cell-attached patch experiments, all sweeps at test potentials of −30 or −20 mV before and after the presence of AII in the bath were analyzed (Table 1). At a test potential of −30 mV, T-type calcium channel open probabilities were 0.023 ± 0.003 in the control and 0.052 ± 0.005 in the presence of 10 nM AII (± SEM; n = 4). In seven control cell-attached patch recordings of single T-type calcium currents not exposed to AII, a similar 2-fold increase of channel activity was not observed (Table 2).

The agonistic effects of AII on T-type calcium channels are apparently mediated through activation of a specific AII receptor since AII-induced enhancement of T-type calcium channels could be prevented by pretreatment of glomerulosa cells with the competitive peptide antagonist of AII, saralasin (Fig. 4*A*).

L-type calcium channel currents were not observed in the patch recording shown in Fig. 3 even at stronger test depolarization to 0 mV. Similar enhancements of single T-type calcium channels as that shown in Fig. 3 were observed in 9 of 11 cell-attached patch recordings mainly obtained at weak test depolarizations that rarely activated L-type calcium channels. The effect of 10 nM AII was tested on three cell-attached patches with detectable L-type channel openings. AII produced no detectable increase in L-type channel activity (evoked from V_h = −30 mV to eliminate any contribution of T-type channels) in any of the three cells tested. The lack of effect of AII on L-type calcium channels was not due to a lack of effect of AII at more depolarizing potentials required to activate L-type channels since enhancement of inward current by AII was also observed with stronger test depolarizations (V_t = −20 mV; Fig. 4*B*). In addition, in one of the cells assessed for effects on L-type calcium channels, the effect of AII was also assessed on T-type calcium current. In this cell AII had no effect on L-type current but markedly increased T-type current.

It is significant that AII added to the bathing solution was able to modulate T-type channels within the patch electrode. Since channels within the pipette are effectively isolated from agonist, the production of a readily diffusible second messenger is required to couple AII receptor activation to T-type calcium channel modulation (11). In other systems a direct receptor–G protein–calcium channel linkage has been proposed to explain the effects of many hormones on voltage-gated calcium channels.

DISCUSSION

By using the patch–voltage clamp technique, it was possible to demonstrate that AII induced an increase of unique T-type single calcium channel activity, low-threshold T-type calcium channel current, and slowly-deactivating T-type calcium channel tail current. This AII-induced enhancement results from a GTP-dependent shift in the voltage dependence of T-type calcium channel activation. Modulation of T-type calcium channels by either AII or atrial natriuretic peptide (2) depends on generation of intracellular second messengers. It is of considerable interest that two important physiological regulators of aldosterone secretion act on this type of calcium channel in unique and specific manners: atrial natriuretic peptide acting via the second messenger cGMP to shift the voltage-dependence of T-type calcium channel inactivation to more negative potentials, and AII acting via an unknown second messenger to shift the voltage-dependence of T-type calcium channel activation to more negative potentials. These observations suggest that T-type calcium channels play an important role in mediating aldosterone secretion (14).

The steroidogenic action of AII is initiated by AII binding to a high-affinity receptor that activates phospholipase C (15). Involvement of G proteins in the activation of phospholipase C has been suggested previously (16). A similar role for G proteins in adrenal glomerulosa cells is likely, since AII-stimulated production of inositol phosphates in glomerulosa

cell membranes is inhibited in the absence of GTP (17). In addition, nonhydrolyzable analogs of GTP enhance phospholipase C activity in these cells (17, 18). The nature of the G protein responsible for augmenting the activity of T-type channels in glomerulosa cells has yet to be determined.

In addition to an involvement of G protein, we have demonstrated that the coupling between the AII receptor and the T-type calcium channel in glomerulosa cells requires the generation of a readily diffusible second messenger. This conclusion is based on the observation that single T-type calcium channel currents within a cell-attached patch are modulated by AII applied to the outside of the patch electrode. Although our experiments do not identify this second messenger, two possible candidates include inositol phosphate and diacylglycerol. Inositol phosphates have been shown previously to modulate other types of membrane ionic channels (19), while the generation of one of several species of diacylglycerol (20) that activate protein kinase C may have a similar role.

Our experiments in bovine adrenal glomerulosa cells were designed to investigate whether AII enhanced T-type calcium channel current. It is important to note, however, that unlike Y-1 cells where both T- and L-type channels appear to be equally expressed (3), T-type calcium channels are the predominant channel type recorded in bovine adrenal glomerulosa cells (1, 2, 21, 22). In our studies T-type channels were recorded in all cell-attached patch recordings ($n = 35$), while L-type channels were recorded in 50% of these recordings. In addition, our whole-cell recordings were often not optimized for recording L-type calcium channel currents. In the absence of pipette solutions designed to slow L-type calcium channel washout (2, 23), the small magnitude of L-type current recorded in whole-cell experiments was rapidly dialyzed away in the 3- to 5-min period allowed to insure dialysis of the cells (1). In contrast, Hescheler *et al.* (3) began their voltage protocols immediately upon disruption of the cell membrane to attain a whole-cell patch and then completed the experiment within 3–5 min. During this brief period of time, the decline in current magnitude of L-type calcium channels was negligibly small (J. Hescheler, personal communication) and relied upon intracellular levels of GTP to not dialyze away. In such experiments, an additional source of GTP could arise from conversion of the large intracellular concentration of ATP to GTP (3). Under these conditions it was presumed that intracellular GTP levels would be sufficient for G-protein activation. Finally, the majority of our studies were designed to maximize T-type calcium channel current and minimize the contribution of L-type channel current. Therefore, we would not definitively rule out an effect of AII on L-type channels, indeed, our single-channel experiments would not have detected an AII-induced modulation of L-type calcium channel currents if mediated by direct G protein-to-channel coupling, as has been observed in cardiac tissue (10). To observe such an effect on L-type channels in a cell-attached patch, AII would have to be present in the patch electrode and would be ineffective when applied only to the bathing solution, as was done in the present experiments.

It has been questioned whether the effects of AII on slow tail currents might have reflected modulation of "mode 2" L-type calcium channel gating rather than T-type channels (4), but the single-channel data presented here leave no doubt that T-type channels are modulated by AII. Whether or not L-type calcium channels are modulated by AII does not offset the conclusion that T-type calcium channels play an important role in the physiological function of glomerulosa cells. This is highlighted by the fact that the activity of T-type calcium channels is modulated by the most important physiological regulator of aldosterone secretion, AII.

This work was supported in part by a grant (DK19813-15) from the National Institutes of Health and was submitted by R.T.McC. in partial fulfillment of requirements for a doctorate in Cell Biology at Yale University.

1. Cohen, C. J., McCarthy, R. T., Barrett, P. & Rasmussen, H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2412–2416.
2. McCarthy, R. T., Isales, C. M., Bollag, W. B., Rasmussen, H. & Barrett, P. (1990) *Am. J. Physiol.* **258**, F473–F478.
3. Hescheler, J., Rosenthal, W., Hinsch, K. D., Wulfern, M., Trautwein, W. & Schultz, G. (1988) *EMBO J.* **7**, 619–624.
4. Hess, P. (1990) *Annu. Rev. Neurosci.* **13**, 337–356.
5. Kojima, I., Kojima, K., Kreutter, D. & Rasmussen, H. (1984) *J. Biol. Chem.* **259**, 14448–14457.
6. Colquhoun, D. (1971) *Lectures on Biostatistics* (Oxford Univ. Press, Oxford).
7. Herrington, J. & Lingle, C. J. (1992) *J. Neurophys.* **68**, 213–232.
8. Lewis, D. L., Weight, F. & Luini, A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9035–9039.
9. Scott, R., Wootton, J. F. & Dolphin, A. C. (1990) *Neuroscience* **38**, 285–294.
10. Yatani, A. & Brown, A. M. (1989) *Science* **245**, 71–74.
11. Neher, E. (1986) in *Membrane Control*, ed. Luttgau, H. C. (Fisher, Stuttgart, F.R.G.).
12. Nilius, B., Hess, P., Lansman, J. & Tsien, R. W. (1985) *Nature (London)* **324**, 473–476.
13. Nowycky, M., Fox, A. & Tsien, R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2178–2182.
14. Barrett, P., Isales, C. M., Bollag, W. B. & McCarthy, R. T. (1991) *Am. J. Physiol.* **261**, F706–F719.
15. Glossman, H., Baukal, A. & Catt, K. (1974) *J. Biol. Chem.* **249**, 664–670.
16. Fleischman, L. F., Chahwala, L. & Cantley, L. (1986) *Science* **231**, 407–409.
17. Guillon, G., Gallo-Payet, N., Balestre, M.-N. & Lombard, C. (1988) *Biochem J.* **253**, 765–775.
18. Enyedi, P. & Williams, G. (1988) *J. Biol. Chem.* **253**, 7940–7942.
19. Kuno, M. & Gardner, P. (1987) *Nature (London)* **326**, 301–304.
20. Bollag, W. B., Barrett, P., Isales, C. & Rasmussen, H. (1991) *Endocrinology* **128**, 231–241.
21. Matsunaga, H., Yamashita, N., Maruyama, Y., Kojima, I. & Kurokawa, K. (1987) *Biochem. Biophys. Res. Commun.* **149**, 1049–1054.
22. Matsunaga, H., Maruyama, Y., Kojima, I. & Hoshi, T. (1987) *Pflügers Arch.* **408**, 351–355.
23. Forscher, P. & Oxford, G. (1985) *J. Gen. Physiol.* **85**, 743–763.