α_1-Adrenoceptors in rat dorsal raphe neurons: regulation of two potassium conductances

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1. α_1-Adrenoceptor activation caused two separate effects in rat dorsal raphe neurons: a depolarization and an increase in the duration of the after-hyperpolarization following the action potential. The depolarization often resulted in repetitive action potentials. The α_1-adrenoceptor antagonists prazosin and WB 4101 blocked the depolarization induced by phenylephrine. The concentration–response curve to phenylephrine was shifted to the right by WB 4101.

2. Under voltage clamp, α_1-adrenoceptor agonists caused an inward current at −60 mV, which often became smaller at negative potentials but rarely reversed polarity even at strongly negative potentials. Using whole-cell recording, the inward current reversed polarity at the equilibrium potential for potassium in the majority of cells. Intracellular Cs^+ decreased or abolished the α_1-mediated inward current. The inward current was dependent on external calcium, but not on the degree of internal calcium buffering. Removal of external calcium or addition of $MgCl_2$, $CoCl_2$ or $CdCl_2$ reduced or blocked the effects of α_1-adrenoceptor agonists. Barium and strontium supported and even augmented the inward current induced by α_1-adrenoceptor agonists, whereas nifedipine and ω-conous toxin had no effect. In contrast, internal dialysis with the calcium chelator 1,2-bis(*O*-aminophenoxy)ethane-*N*,*N*,*N*′*N*′-tetraacetic acid (BAPTA) did not inhibit the inward current.

3. The α_1-induced depolarization was blocked (or occluded) by the inclusion of GTP-γ-S (100 μM) in the recording pipette. The phorbol-ester 4-phorbol 12,13-dibutyrate (PDBu) had no action on the membrane potential and depressed the phenylephrine-induced depolarization. This depression was reversed by the non-selective protein kinase inhibitor staurosporin.

4. Phenylephrine and noradrenaline increased a late component of the after-hyperpolarization (late-AHP) that followed a single action potential. The α_1-sensitive late-AHP was blocked by apamine suggesting that it is a calcium-dependent potassium conductance.

5. Thapsigargin reduced the duration of the late-AHP and blocked the phenylephrine-mediated prolongation. Caffeine also augmented the late-AHP and ryanodine blocked the augmentation induced by caffeine. The augmentation induced by phenylephrine was not occluded by caffeine and was still present after the caffeine-induced augmentation was blocked by ryanodine.

6. In slices pretreated with manoalide the depolarization induced by α_1-agonists was not changed; however, the late-AHP was reduced in duration and the α_1-receptor-mediated augmentation of the late-AHP was decreased.

7. The results suggest that the depolarization of dorsal raphe neurons by α_1-adrenoceptor activation is through a decrease in potassium conductance that is independent of the activation of the phospholipase C pathway. The augmentation of the late-AHP is mediated by release of calcium from intracellular stores and may serve to regulate activity during the depolarization induced by α_1-adrenoceptor activation.

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There are several sites in the central nervous system where the activation of α_1-adrenoceptors has been found to increase excitability. Included among these sites are the neurons of the dorsal raphe nucleus (Yoshimura, Higashi & Nishi, 1985), where the firing rate is thought to be regulated by noradrenergic tone (Baraban & Aghajanian, 1980). Electrical stimulation in the area of the dorsal raphe in brain slices evoked a slow excitatory postsynaptic potential mediated by noradrenaline acting on α_1-adrenoceptors (Yoshimura *et al.* 1985). In addition, amphetamine, an agent that releases noradrenaline, caused a depolarization that was blocked by prazosin, indicating that the release of endogenous noradrenaline increased the excitability of dorsal raphe neurons (Pan & Williams, 1989). The most commonly reported ionic mechanism that mediates the depolarization induced by α_1-adrenoceptor activation is a decrease in potassium conductance (Yoshimura *et al.* 1985; Fukuda, Minami, Nabekura & Oomura, 1987; Freedman & Aghajanian, 1987; McCormick & Prince, 1988; Legendre, Dupouy & Vincent, 1988; McCormick, 1992; Larkman & Kelly, 1992). There are several additional reports of noradrenaline acting on various conductances, ranging from an inhibition of the transient potassium conductance (Aghajanian, 1985; Wang, Wettwer, Gross & Ravens, 1991) to both augmentation (Freedman & Aghajanian, 1987) and depression (Yoshimura, Polosa & Nishi, 1986, 1987) of the conductances underlying the after-hyperpolarization following the action potential. Although activation of α_1-adrenoceptors is known to increase phosphatidylinositol turnover (Burch, Luini & Axelrod, 1987; Johnson & Minneman, 1987), the second messenger pathway mediating neuronal depolarization is still poorly understood. The purpose of this study was to identify the effects mediated by α_1-adrenoceptor stimulation in dorsal raphe neurons and to investigate the possible second messengers involved in this depolarization. Some of this work has been presented in an abstract (Grudt & Williams, 1990).

METHODS

Intracellular recordings were made from dorsal raphe cells in brain slices from adult Wistar rats. The methods employed were similar to those published previously (Williams, Colmers & Pan, 1988). Briefly, animals were anaesthetized with halothane and killed by decapitation, and the brain was removed. Brain slices (300 μm thick) were cut in a vibratome in cold (4 °C) physiological saline. Two or three coronal slices were taken near where the aqueduct opened into the fourth ventricle. A single slice was submerged in a tissue bath through which flowed physiological saline (1·5 ml min^{-1}) at 35 °C. The content of physiological saline solution was (mM): NaCl, 126; KCl, 2·5; NaH_2PO_4, 1·2; $MgCl_2$, 1·2; $CaCl_2$, 2·4; glucose, 11; $NaHCO_3$, 25; gassed with 95% O_2–5% CO_2 at 37 °C. The area of the dorsal raphe was recognized in the slice as a triangular area in the mid-line just above medial lemniscus. Intracellular recordings were made with glass microelectrodes having resistances of 40–70 MΩ. Membrane currents were recorded with a single-electrode voltage-clamp amplifier (Axoclamp 2A, Axon Instruments, Foster City, CA, USA) using switching frequencies between 3 and 6 kHz. The settling time of the clamp following a 10 mV step was typically 3–5 ms. Steady-state current–voltage (I–V) plots were constructed with two methods. First, slow depolarizing potential ramps were applied at a speed of (1 mV s^{-1}) and the current was recorded directly on an x–y plotter. Second, currents were measured at the end of 500 ms voltage steps to different potentials. Both methods gave identical results. Whole-cell recordings were made with an Axopatch 1D amplifier using the blind patch method. Unless otherwise stated, the recording pipettes (resistance 3–5 MΩ) were filled with a solution containing (mM): potassium gluconate, 126; NaCl, 10; $MgCl_2$, 1; EGTA, 11; ATP, 2; GTP, 0·25; and Hepes, 10; pH was adjusted to 7·3 with NaOH. Seal resistances were typically 5 GΩ or above. Whole-cell recordings with an access resistance of less than 10 MΩ were considered acceptable and series resistance was compensated by at least 80%. All whole-cell experiments were done in solution containing tetrodotoxin (TTX, 1 μM). In all experiments, drugs were applied by superfusion at the indicated concentration.

Materials

The following drugs and salts were used: TTX (Sigma, St Louis, MO, USA), DL-2-amino-5-phosphonovaleric acid (AP-5; Sigma), 6-cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX; Tocris Neuramin, Bristol, UK), kynurenic acid (Sigma), (−)bicuculline methiodide (Sigma), prazosin (Research Biochemicals Inc., Natick, MA, USA), 5-hydroxytryptamine (5-HT; Sigma), 5-carboxamidotryptamine (5-CT; Research Biochemicals Inc.), 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)-butyl]-piperazine hydrobromide (NAN-190; Research Biochemicals Inc.), spiperone (Research Biochemicals Inc.), ketanserin (Sigma), (3-tropanyl)-1-H-indole-3-carboxylic acid ester (ICS 205–930; Research Biochemicals Inc.), manoalide (Biomol, Plymouth Meeting, PA, USA), ryanodine (Calbiochem, LaJolla, CA, USA), caffeine (Sigma), phenylephrine (PE; Sigma), noradrenaline (NA; Sigma), WB 4101 (Research Biochemicals Inc.), apamine (Sigma), thapsigargin (Calbiochem), staurosporin (Kamiya Biomedical Co., Thousand Oaks, CA, USA), 4-phorbol 12, 13-dibutyrate (PDBu), 1,2-bis(*O*-aminophenoxy)ethane-*N*,*N*,*N*′*N*′-tetraacetic acid (BAPTA), picrotoxin, strychnine, ATP, GTP and GTP-γ-S (all from Sigma). Numerical data are presented as means ± S.E.M. and compared using Student's paired or unpaired, two-tailed t test.

RESULTS

α_1-Adrenoceptor activation caused a depolarization

Noradrenaline depolarized dorsal raphe neurons and often caused the generation of repetitive action potentials (Fig. 1*A*). The amplitude of the depolarization was dependent on the concentration of noradrenaline applied; however, no quantitative experiments were carried out with noradrenaline as it is a substrate for reuptake. Phenylephrine, a selective α_1-adrenoceptor agonist, caused a concentration-dependent depolarization that had an EC_{50} of 1·4 μM (Fig. 2). The peak amplitude of the depolarization induced by phenylephrine (10 μM) was

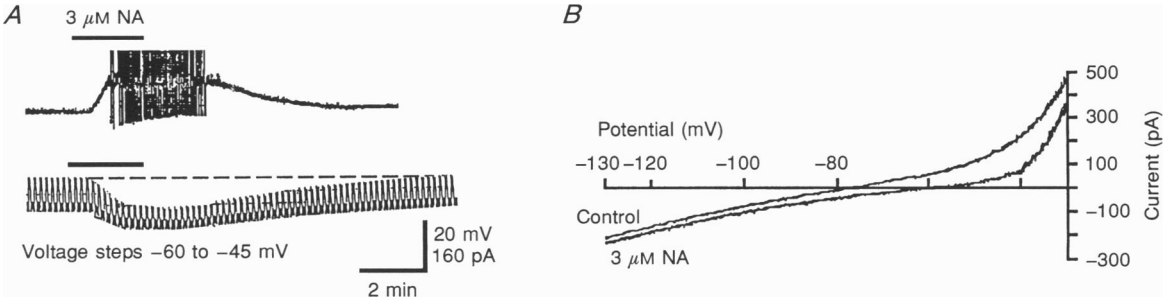

Figure 1. Noradrenaline depolarizes dorsal raphe neurons and decreases membrane conductance
Recordings made with an intracellular electrode. *A*, top trace, a recording of membrane potential. In this and other recordings the bar above the trace indicates the period that the superfusion solution was changed to one containing the drug at the indicated concentration. Noradrenaline (NA) depolarized this neuron about 15 mV from the resting potential of −65 mV. Lower trace, a current recording from the same cell as shown above. The holding potential was stepped from −60 to −45 mV throughout the recording. Noradrenaline caused an inward current that was larger at −45 mV than at −60 mV. *B*, two superimposed current–voltage (*I–V*) traces, one taken before (Control) and the other during the superfusion with noradrenaline (3 μM). Traces were obtained directly from an *x–y* plotter and during a voltage ramp. Noradrenaline caused an inward current throughout the voltage range tested.

$14{\cdot}9 \pm 1{\cdot}0$ mV ($n = 27$). There was no evidence for desensitization to phenylephrine (100 μM); the amplitude of the depolarization was steady over a 30 min application period (Fig. 2*A*, $n = 5$). The depolarization induced by noradrenaline and phenylephrine was completely blocked by prazosin (100 nM, $n = 5$). In addition, the concentration–response curve to phenylephrine was shifted 6-fold to the right by the α_1-adrenoceptor antagonist WB 4101 (100 nM), resulting in an estimated K_d of 20 nM (Fig. 2*B*).

α_1-Adrenoceptor-mediated inward current

Potassium conductance

Under voltage clamp, noradrenaline produced an inward current throughout the voltage range tested (Fig. 1*B*). The inward current induced by α_1-adrenoceptor activation was the largest at about −40 mV and declined as the membrane potential was shifted to more negative potentials (Fig. 1*B*). In the majority of cells (18/30) recorded using intracellular electrodes, noradrenaline or phenylephrine caused an

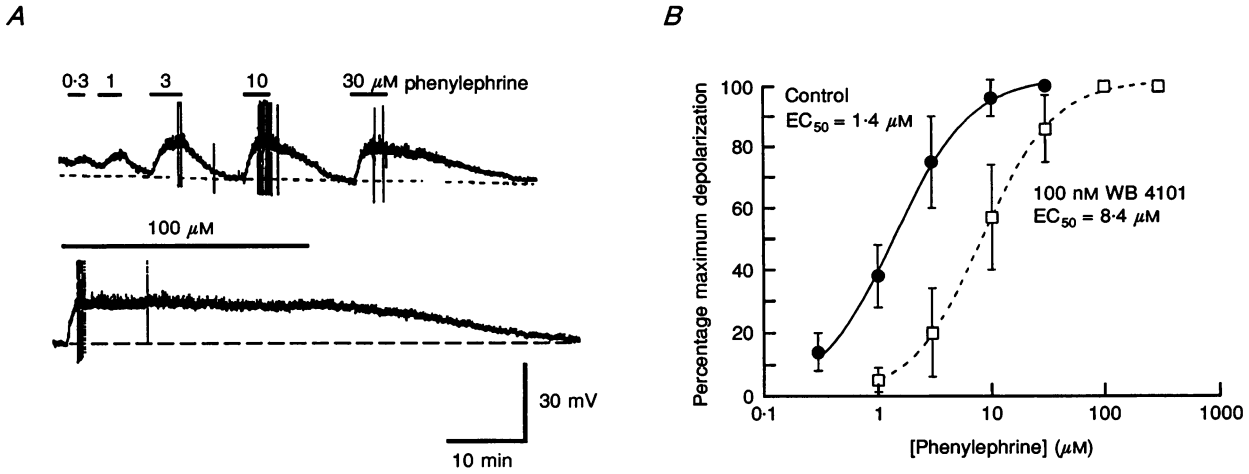

Figure 2. Phenylephrine depolarized dorsal raphe neurons in a concentration-dependent manner through activation of an α_1-adrenergic receptor
A, a voltage recording showing a typical concentration-dependent depolarization induced by phenylephrine. The dashed line indicates −67 mV. Lower trace, during a prolonged application of a high concentration of phenylephrine the depolarization was maintained. *B*, concentration–response curve for phenylephrine in control and in the presence of WB 4101 (100 nM). Each cell was normalized to its own maximum depolarization (30 μM in control and 100 μM in WB 4101, $n = 3$). Error bars show the standard deviation of the mean.

inward current, even at strongly negative potentials (more negative than −120 mV, Fig. 1*B*). In other cells (12/30) the noradrenaline current declined to zero at strongly negative potentials but did not reverse polarity. When the external potassium concentration was increased to 10·5 mM, there was no change in the ratio of cells that had inward currents at strongly negative potentials (5/7) and there were no cells where the phenylephrine current reversed polarity.

With whole-cell recording, noradrenaline or phenylephrine caused a depolarization or an inward current in every cell tested ($n = 60$). In the majority of cells (33/50), the slope conductance between −60 and −70 mV increased from 3·7 ± 0·7 nS at the start of the recording (within the first 5 min) to 12·7 ± 0·6 nS after 15 min. When phenylephrine was tested within 5 min, the results were similar to those obtained with intracellular electrodes, that is, phenylephrine caused an inward current that did not reverse polarity even at strongly negative potentials. After 15–20 min, the conductance of the cell had increased and become steady. Phenylephrine then caused an inward current at rest that reversed polarity (Fig. 3*A*). The reversal potential of this current was −92 ± 2 mV ($n = 33$), which was similar to the reversal potential of the 5-HT-induced potassium current (−94 ± 3 mV, $n = 10$). The reversal potential of the phenylephrine-induced current was shifted to less negative potentials in solutions of elevated potassium and gave a Nernst slope of −47 ± 4 mV per 10-fold change in potassium concentration (Fig. 3*B* and *C*).

The other seventeen cells were distinguished by the relative stability of the membrane conductance (2·5 ± 0·5 nS in the first 5 min to 4·4 ± 0·5 nS after 15 min). Phenylephrine caused an inward current that declined at negative potentials but did not reverse polarity in these cells. This

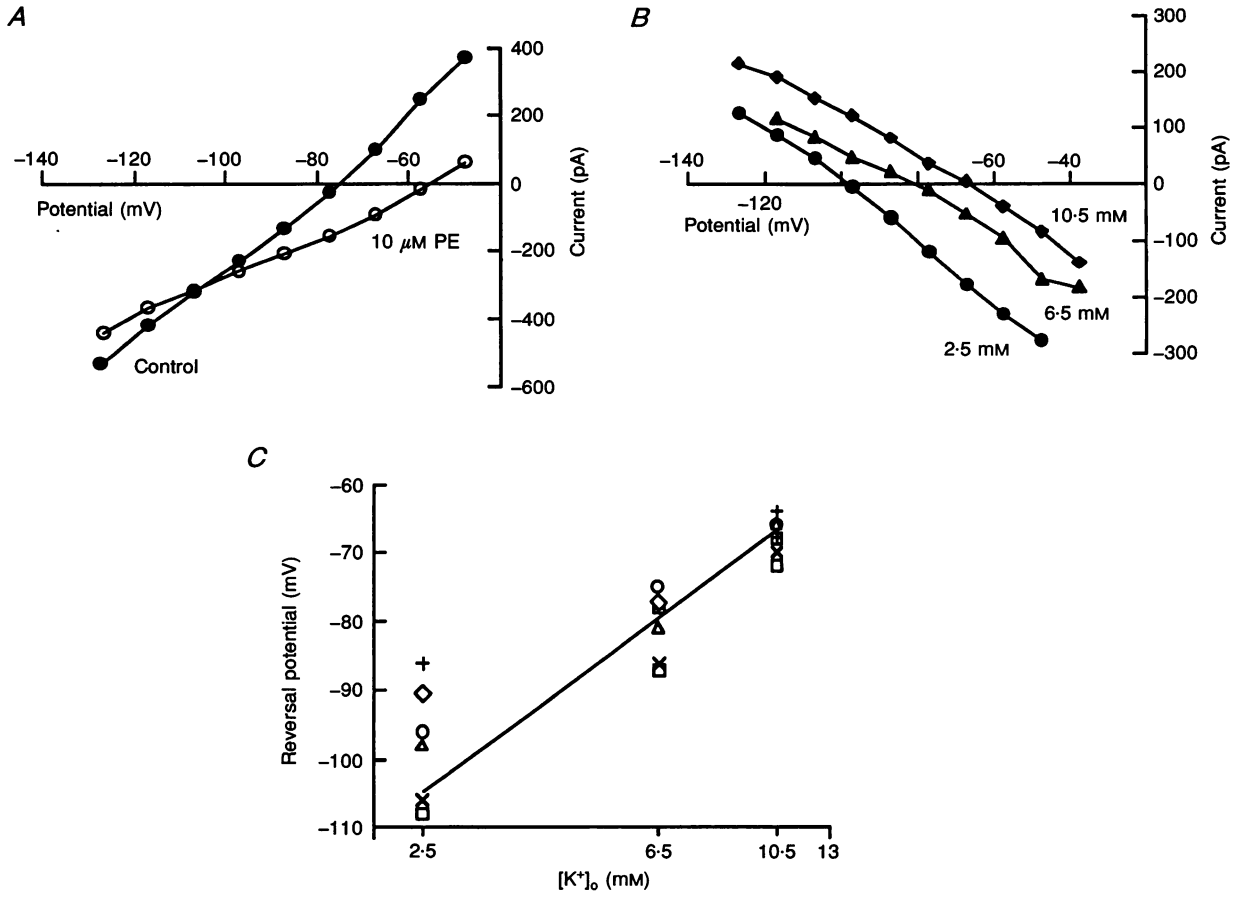

Figure 3. The inward current caused by phenylephrine using whole-cell recording is potassium dependent

A, *I–V* plots from a cell in the absence (Control) and presence of phenylephrine (PE; 10 μM). The cell was held at −60 mV and voltage steps were applied (400 ms, every 2 s) to potentials ranging from −50 to −130 mV. PE caused a net inward current at −60 mV that reversed polarity at −110 mV. *B*, the reversal potential of the PE current is dependent on the concentration of extracellular potassium. PE-induced currents are plotted as a function of the membrane potential. These traces were obtained by subtraction of the *I–V* in control from the *I–V* in PE. *C*, Nernst plot of reversal potentials against three potassium concentrations in each of 6 cells. The line is calculated from the Nernst equation for a potassium-selective electrode.

may be a second cell type since the recording conditions, electrodes and access resistances were identical with recording from the other cells and were not studied further.

The inward current induced by α_1-adrenoceptor activation was occluded by caesium applied internally after 15–30 min of equilibration. With both intracellular and whole-cell recordings, the inward current induced by both noradrenaline and phenylephrine was not present in four of seven cells using intracellular electrodes and in three of three cells using whole-cell recording.

G protein dependence

Inclusion of GTP-γ-S (100 μM, with normal GTP and ATP) in the whole-cell recording pipette caused a hyperpolarization that began within 2–4 min and reached a plateau at about −90 mV within 10 min. Once the membrane potential had become steady, superfusion with phenylephrine (10 μM) had no effect ($n = 3$). In these same cells, superfusion with 5-carboxamido tryptamine (5-CT, 1 μM), a 5-HT_{1A} agonist that increases potassium conductance through a pertussis toxin-sensitive G protein (Williams *et al.* 1988), also failed to affect membrane potential. It was previously shown that the α_1-adrenoceptor-mediated depolarization was not blocked by pertussis toxin treatment (Williams *et al.* 1988; McCormick, 1992).

Calcium dependence

Using both intracellular and whole-cell recording methods, the inward current induced by α_1-agonists was reduced or abolished by solutions containing low calcium (1 mM) and high magnesium (10 mM), cobalt (5 mM) or cadmium (100–300 μM, Fig. 4). The phenylephrine-induced current at −60 mV was 124 ± 30 pA in control and 27 ± 10 pA in $CdCl_2$ (100–300 μM). These calcium channel blockers often caused an inward current themselves, such that it was difficult to determine if their action was to simply occlude the inward current induced by the α_1-adrenoceptor agonists. Similar results have been reported in cultured spinal cord cells (Legendre *et al.* 1988). Neither nifedipine (1–3 μM, $n = 4$) nor ω-conus toxin (300 nM, $n = 3$) had any action by themselves or on the α_1-adrenoceptor-mediated inward current. In contrast to the divalent cations that block calcium currents, the inward current induced by phenylephrine was still present in solutions where $BaCl_2$ (2 mM) and $SrCl_2$ (2 mM) were added to the normal calcium-containing solution. In addition, the phenylephrine-induced current obtained in solutions where strontium was substituted for calcium was 2·7 ± 0·3-fold larger ($n = 6$) at −60 mV than in control solutions. The observation that only divalent cations that non-selectively block calcium

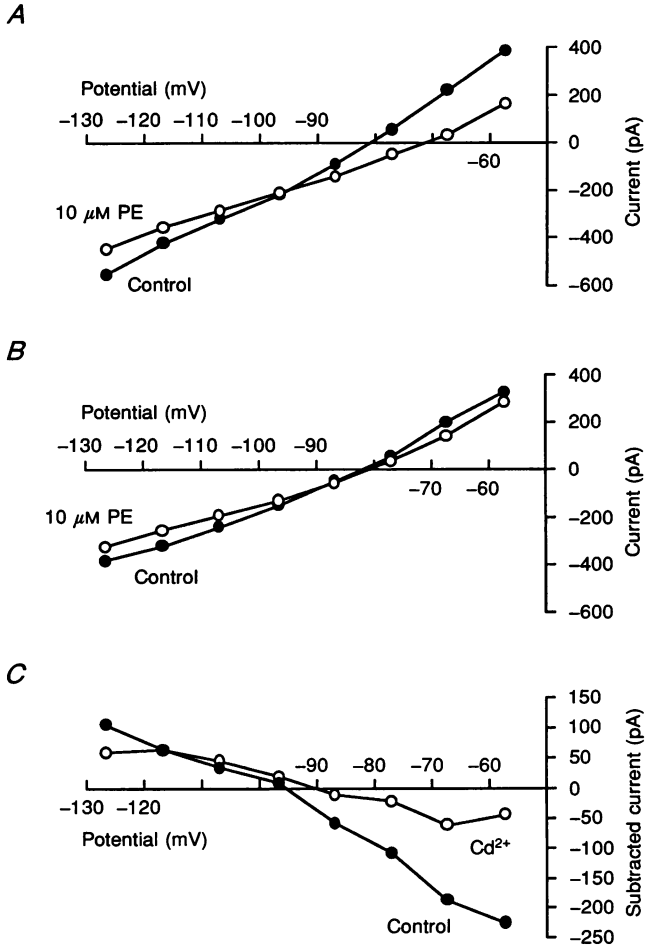

Figure 4. The whole-cell inward current induced by phenylephrine is reduced by cadmium (100 μM)
A, two superimposed *I–V* plots, one in control and the other in the presence of PE (10 μM) in normal solution. *B*, in the presence of $CdCl_2$ (100 μM), the slope conductance of the cell is decreased and the current induced by PE is reduced. *C*, the PE current in the presence of Cd^{2+} is reduced at all membrane potentials. The subtracted currents from the traces in *A* and *B* are plotted as a function of membrane potential.

channels decrease the α_1-adrenoceptor-mediated currents suggests that calcium may play an important role in the phenylephrine effect.

The dependence of the α_1-induced inward current on external calcium prompted experiments using whole-cell recording with pipettes containing solutions having differing calcium buffering capacity. Responses to phenylephrine were compared using solutions having either high calcium buffering capacity (20 mM BAPTA, no added calcium) or low calcium buffering capacity (1 mM EGTA + 100–400 μM calcium; free calcium 10–50 nM at pH 7·3). When the BAPTA internal solution was used, phenylephrine caused a depolarization of 15 ± 2 mV ($n = 5$), similar in amplitude to that observed with normal EGTA internal solution (13 ± 2 mV, $n = 4$). In voltage clamp, however, the phenylephrine-induced current did not reverse polarity at negative potentials ($n = 6$).

Using internal solutions of low calcium buffering capacity, we found no significant difference in the resting conductance or in the inward current induced by phenylephrine from results obtained with the normal EGTA internal solution.

α_1-Adrenoceptor activation prolongs the late-AHP

In addition to the depolarization, phenylephrine ($n = 14$) and noradrenaline ($n = 3$) increased the amplitude and duration of a late after-hyperpolarization (late-AHP) following a single action potential (Fig. 5). Action potentials were evoked from the same potential (−75 mV) in the presence and absence of phenylephrine or noradrenaline. The action potential and the early part of the after-hyperpolarization were not changed by either agonist. The late-AHP had a peak amplitude of $13 \pm 0{\cdot}4$ mV that occurred 54 ± 13 ms after the onset of the action potential and had a time constant of decay ranging from 80 to 150 ms ($n = 4$). In phenylephrine, the amplitude increased by $1{\cdot}38 \pm 0{\cdot}06$-fold, the time to peak increased $1{\cdot}4 \pm 0{\cdot}15$-fold and the time constant of decay increased $1{\cdot}95 \pm 0{\cdot}16$-fold ($n = 4$; Fig. 5*B*). Apamine (100–300 nM) blocked the late-AHP in all three cells tested (Fig. 5*A*). The depolarization induced by phenylephrine persisted in apamine; however, the effect on the amplitude of the late-AHP was reduced to less than 20% of control ($n = 3$).

Dependence on internal calcium stores

To investigate further the mechanism for the increase in the late-AHP by phenylephrine we used thapsigargin and ryanodine, agents known to disrupt the regulation of internal stores of calcium. Thapsigargin decreases non-selectively the ability of agonists to release calcium stores (Baro & Eisner, 1992; Thuringer & Sauve, 1992), whereas ryanodine selectively affects a caffeine-sensitive store of calcium (Marrion & Adams, 1992). The actions of thapsigargin (1–3 μM), ryanodine (10 μM) and caffeine (10 mM) were investigated using intracellular recordings to determine the involvement of intracellular Ca^{2+} stores in the α_1-adrenoceptor prolongation of the late-AHP.

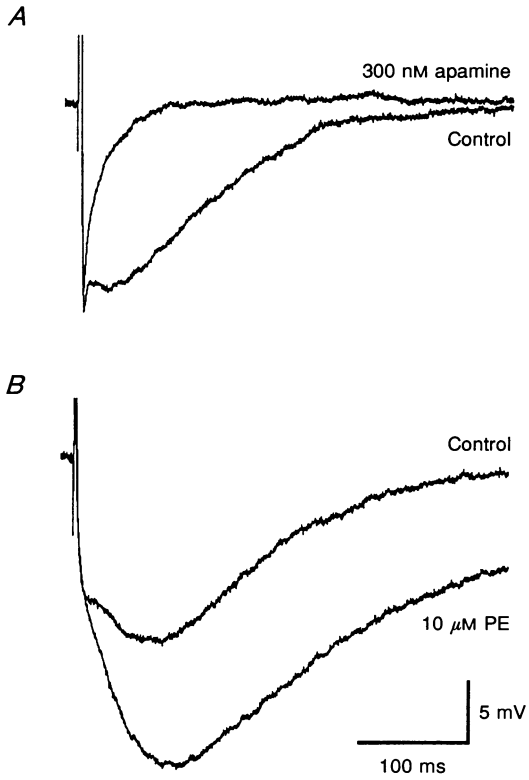

Figure 5. Apamine blocks and phenylephrine augments the late-AHP
In this and other recordings the action potential has not been reproduced. The after-hyperpolarization is made up of fast and slow components. *A*, two superimposed voltage traces of four averaged after-hyperpolarizations. Apamine blocked the late-AHP. *B*, another cell where superfusion with phenylephrine (PE, 10 μM) augmented the late-AHP.

Table 1. Caffeine and PE modulate the late-AHP through different internal Ca^{2+} stores

		Control	Caffeine (10 mM)	Control	PE (10 μM)
Control	*A*	11·6 ± 1·0 (10)	14·3 ± 1 (10)***	11·5 ± 1 (6)	15 ± 0·8 (6)**
	τ	102 ± 11 (10)	205 ± 71 (10)**	96 ± 10 (6)	157 ± 20 (6)**
Thapsigargin (1–3 μM)	*A*	8·3 ± 0·4 (3)	8·4 ± 0·3 (3)	6·8 ± 0·3 (3)	7·1 ± 0·1 (3)
	τ	62 ± 3 (3)	70 ± 7 (3)	62 ± 5 (3)	90 ± 6 (3)
Ryanodine (10 μM)	*A*	9·9 ± 0·6 (7)	9·9 ± 0·6 (7)	10·9 ± 0·8 (11)	13·0 ± 1·1 (11)**
	τ	73 ± 4 (7)	89 ± 7 (7)	82 ± 5 (11)	168 ± 18 (11)***

Action potentials were evoked from a membrane potential of −75 mV. *A*, amplitude (mV); *τ*, time constant of decay (ms). Data presented as means ± S.E.M., with values of *n* shown in parentheses. $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

Caffeine (10 mM), like phenylephrine, increased the amplitude, duration and the time to peak of the late-AHP (Table 1, $n = 10$).

Thapsigargin (1–3 μM) reduced the amplitude and time course of the late-AHP (Table 1, Fig. 6*A*). Ryanodine (10 μM) also significantly decreased the time constant of decay of the late-AHP (Table 1, $P < 0{\cdot}03$, $n = 9$, Fig. 7*A*). Thapsigargin blocked both the caffeine and phenylephrine-induced augmentation of the late-AHP (Table 1, Fig. 6*B*). Ryanodine (10 μM for 10 min) blocked the prolongation induced by caffeine (Table 1, Fig. 7*B* and *C*, $n = 10$) but not phenylephrine (Table 1, Fig. 8*A*, $n = 11$).

In another group of cells ($n = 5$), phenylephrine was tested in the presence of caffeine. After the late-AHP was increased by caffeine, phenylephrine caused a further increase in the late-AHP (Fig. 8*B*). None of the compounds tested – thapsigargin, ryanodine or caffeine – had a significant effect on the resting membrane potential or the α_1-adrenoceptor-induced depolarization. The phenylephrine depolarization was 11·7 ± 0·3 mV in control, 12·0 ± 0·6 mV in thapsigargin, 10·0 ± 0·6 mV in ryanodine and 9·3 ± 1·8 mV in caffeine. These results indicate that caffeine augments calcium release from a ryanodine-sensitive store and that α_1-adrenoceptors act to augment the late-AHP through a separate pool of calcium.

Dependence on the activation of phospholipase C

α_1-Adrenoceptors have been shown to activate phospholipase C (PLC) to increase production of inositol 1,4,5-trisphosphate (IP_3) and consequently release internal stores of Ca^{2+}. The effect of a PLC blocker, manoalide, on the late-AHP was investigated. Prolonged treatment with

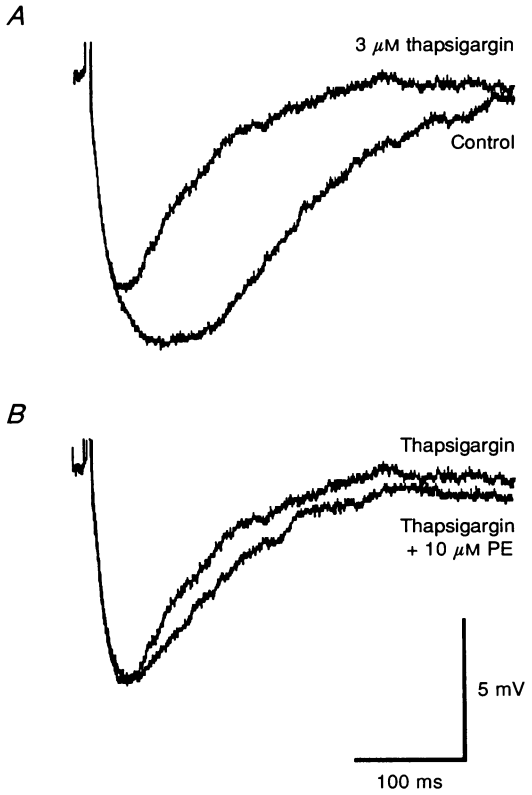

Figure 6. Thapsigargin reduced the late-AHP and blocked the augmentation caused by phenylephrine (PE, 10 μM)
A, the late-AHP is reduced by thapsigargin (3 μM, for 40 min). *B*, same cell as in *A*, the augmentation of the late-AHP was blocked following treatment with thapsigargin.

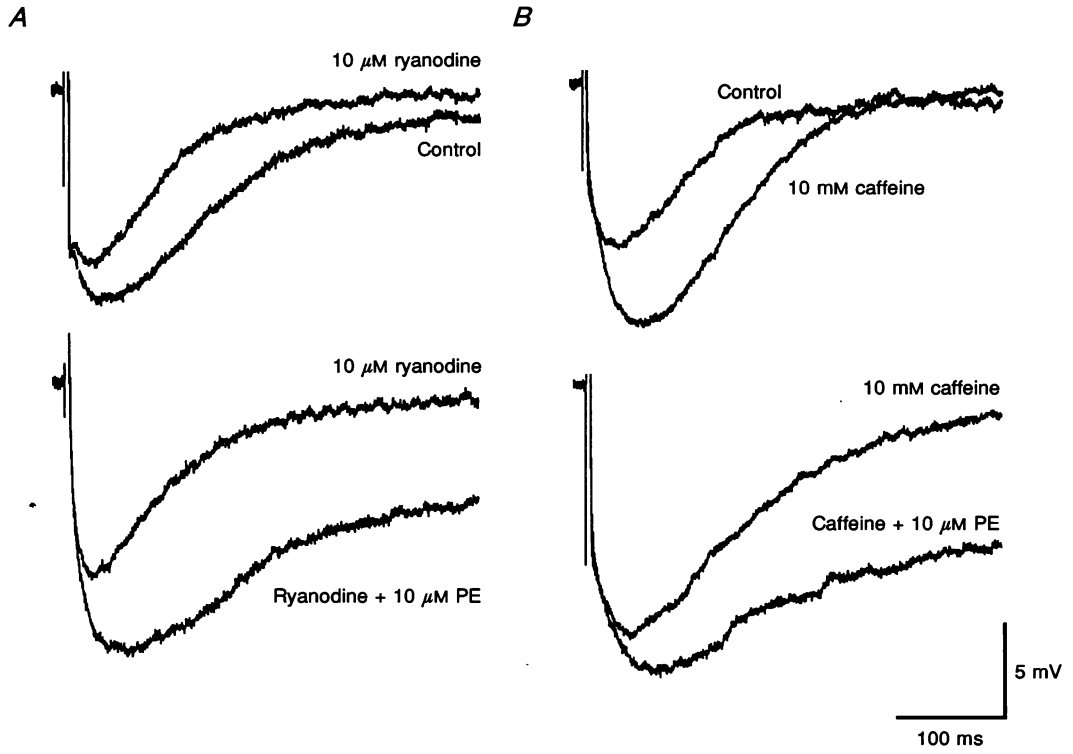

Figure 7. The augmentation of the late-AHP by phenylephrine is independent of the action of ryanodine

A, the augmentation induced by phenylephrine was still present following treatment with ryanodine. *B*, the augmentations induced by phenylephrine and caffeine were additive.

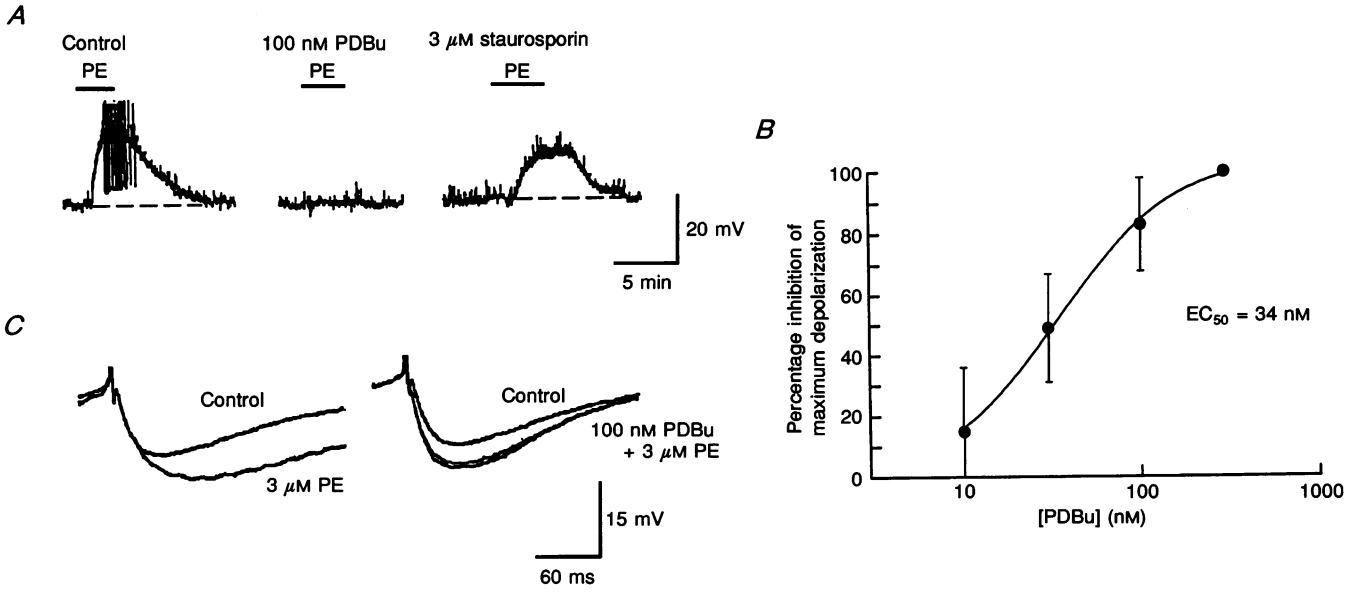

Figure 8. Phorbol esters block α_1-adrenergic receptor-mediated actions

A, left side, in control phenylephrine causes a depolarization and evoked action potential generation. Middle, in the presence of PDBu (100 nM) the PE-induced depolarization was completely blocked. Right side, addition of staurosporin (3 μM) for 20 min partially reversed the blockade induced by PDBu. *B*, concentration–response curve for the inhibition of the PE (3 μM)-induced depolarization by increasing concentrations of PDBu in three cells. Error bars are the standard deviation of the mean. *C*, the augmentation of the late-AHP by phenylephrine (3 μM) was blocked by PDBu. Note that PDBu has a qualitatively different action on the amplitude of the AHP.

manoalide was required to observe an effect such that groups of cells had to be compared rather than each cell acting as its own control. The most consistent effect of manoalide (10 μM, 60 min pretreatment) on the late-AHP was a decrease in the time constant of decay 127 ± 8 ms in control to 87 ± 12 ms after manoalide ($P < 0.02$). In control, the AHP consisted of two components. With the decrease in the time constant of decay in cells treated with manoalide, the separation of the two components was less distinct. In addition, the enhancement of the amplitude of the late-AHP by phenylephrine was reduced. In control, PE significantly increased the amplitude of the late-AHP (13·0 ± 0·3 mV to 18·2 ± 0·5 mV, $P < 0.01$, $n = 5$) and this action was blocked after pretreatment with manoalide (10·3 ± 1·8 mV before and 11·4 ± 2·0 mV after, NS). The α_1-adrenoceptor-induced depolarization was not changed following pretreatment of slices with manoalide (13·6 ± 1·3 mV in control and 15·8 ± 1·8 mV after manoalide). The results suggest that the prolongation of the AHP may be mediated via IP_3, or an effector pathway distinct from that involved in the α_1-mediated depolarization.

Phorbol esters inhibit α_1-adrenceptor activation

Under control conditions, superfusion with phorbol 12,13-dibutyrate (PDBu; 100 nM–10 μM) produced an increase in spontaneous synaptic potentials and a depolarization of 7–15 mV in all three cells tested (intracellular recording). The inactive phorbol ester phorbol 12,13-didecanoate did not increase spontaneous synaptic potentials and had no effect on membrane potential ($n = 4$). Although PDBu apparently mimicked the depolarization induced by phenylephrine, PDBu (10 nM–3 μM) had no effect on the membrane potential in the presence of kynurenate (500 μM), CNQX (10 μM), picrotoxin (100 μM), strychnine (100 nM) and cyanopindolol (1 μM; $n = 10$). These antagonists block glutamate, $GABA_A$, glycine and 5-HT_{1A} receptors, suggesting that the depolarization induced by PDBu was secondary to the release of transmitters.

Although there was no depolarization induced in the presence of the antagonists, low concentrations of PDBu depressed the depolarization induced by phenylephrine (3 μM, Fig. 8*A*). The depression of the phenylephrine depolarization was dependent on the concentration of PDBu applied having an EC_{50} of 34 nM (Fig. 8*B*) and a complete suppression was obtained at concentrations above 100 nM. The kinase inhibitor staurosporin (3 μM) reversed the PDBu-induced inhibition of the phenylephrine depolarization (Fig. 8*A*, $n = 3$), suggesting that PDBu was activating C-kinase to disrupt the α_1-adrenoceptor-mediated depolarization. Staurosporin (3 μM) alone had no effect on the membrane potential ($n = 7$), nor did it change the phenylephrine depolarization ($n = 2$). PDBu (100–300 nM) increased the amplitude of the early part of the after-hyperpolarization following the action potential in two of three cells and completely suppressed the prolongation of the late-AHP induced by phenylephrine in both cells tested (Fig. 8*C*).

DISCUSSION

Activation of α_1-adrenoceptors results in two separate effects on the excitability of dorsal raphe neurons, a depolarization and an augmentation of the late-AHP. These effects are mediated by distinct ionic mechanisms and second messenger pathways.

The depolarization is due to a potassium conductance decrease

Activation of α_1-adrenoceptors depolarizes dorsal raphe neurons. The primary ionic mechanism for this depolarization appears to be through a decrease in potassium conductance. There is an additional action that prevents the observation of a reversal in polarity of the current at the potassium equilibrium potential found with intracellular recordings and within the first 5 min of whole-cell recordings. One possibility for the inability to obtain a reversal potential is an inadequate space clamp of these cells in the slice preparation. The inability to demonstrate a reversal potential is similar to the results obtained in cultured spinal cord cells (Legendre *et al.* 1988) and thalamic relay neurons (McCormick & Prince, 1988) where reversal potentials were estimated by extrapolation. McCormick (1992) obtained reversal potentials of the noradrenaline-induced current at the potassium equilibrium potential in brain slices containing dorsal lateral geniculate neurons. In addition, the noradrenaline-induced current in isolated facial motoneurons also reversed polarity at the potassium equilibrium potential (Larkman & Kelly, 1992). With whole-cell recording, the conductance of about 60% of cells increased with time, after which phenylephrine caused an inward current that reversed polarity at the potassium equilibrium potential. It is possible that the space clamp changed after 15 min of whole-cell recording. It is also possible that with whole-cell recording there is a run-down (or wash-out) of a factor that prevents the observation of a reversal potential. Reversal potentials of the phenylephrine current after 15 min were not obtained with BAPTA in the pipette, suggesting BAPTA prevented the wash-out of a second response that was observed with EGTA in the pipette. Taken together, the results indicate that one component of the phenylephrine-induced depolarization is due to a decrease in potassium conductance, but a second more labile component may also exist.

The depolarization is sensitive to external calcium

The depolarization and associated inward current induced by α_1-adrenoceptor agonists were blocked by the non-selective calcium channel blockers Mg^{2+}, Co^{2+} and Cd^{2+} but not nifedipine or ω-conous toxin. The non-selective

blockers caused an inward current that may have occluded the receptor-mediated inward current through depression of a calcium-dependent potassium current. However, the α_1-mediated current was not blocked by other agents that block calcium-activated potassium conductance – Ba^{2+} (2 mM), Sr^{2+} (2 mM), apamine (100–300 nM) and TEA (10 mM). Similar results have been obtained in experiments from spinal neurons in culture (Legendre *et al.* 1988). Our results differ from other reports on neurons in the dorsal raphe that indicate that the major excitatory effects of phenylephrine in the dorsal raphe is not calcium dependent (Aghajanian, 1985; Freedman & Aghajanian, 1987). The calcium dependence of the α_1-mediated inward current appears to only involve external calcium. This has been reported for the activation of inositol phosphate turnover in brain slices (Johnson & Minneman, 1987), HeLa cells expressing both α_{1C}- and α_{1B}-adrenoceptors (Schwinn *et al.* 1991) and for arachidonic acid in FRTL5 thyroid cells (Burch, Luini & Axelrod, 1986). In the present study, we used BAPTA (20 mM) to buffer internal calcium in order to reduce internal free calcium to very low levels. Although this solution itself had strong hyperpolarizing effects on the cells, the depolarization or inward current induced by phenylephrine was not blocked. This observation indicates that the inward current is not dependent on a generalized release of calcium from internal stores or the calcium-dependent translocation of enzymes to the plasma membrane. The calcium dependence suggests that either some external event, such as binding of agonist, is calcium dependent or that calcium entry into a highly compartmentalized pool near the membrane is required.

The late-AHP, α_1-adrenoceptor activation and the phospholipase C pathway

Activation of α_1-adrenoceptors is known to result in the activation of phospholipase C and thus a release of IP_3 and DAG (Berridge & Irvine, 1989; Huang, 1989; Berridge, 1993). We were interested to determine if this pathway was involved in the α_1-mediated effects in dorsal raphe neurons. Application of the phorbol ester PDBu presumably activated protein kinase C and blocked the α_1-mediated depolarization. Staurosporin, a protein kinase inhibitor, blocked the inhibition by PDBu but had no effect by itself on the α_1-mediated depolarization suggesting that kinases may not be directly involved. The release of DAG with the subsequent activation of protein kinase C may serve as a mechanism to desensitize the receptor (Burch *et al.* 1986). Under our experimental conditions, however, we did not see evidence for desensitization of the α_1-mediated depolarization even with continued application of high agonist concentrations. It is possible that there are additional effects of α_1-adrenoceptor activation that would limit the desensitization (e.g. activation of a phosphatase) that would not be observed with the activation of protein kinase C by phorbol esters.

The depolarization induced by α_1-adrenoceptor activation was not changed after treatment with manoalide, a phospholipase inhibitor. However, the late-AHP itself and the prolongation of the late-AHP by α_1-agonists were affected. Although non-specific effects of manoalide cannot be ruled out, these results suggest that the late-AHP and the augmentation by α_1-adrenoceptor agonists is dependent on a phospholipase pathway. Manoalide caused a qualitative change in the shape of the late-AHP by selectively reducing the late component. The prolongation of the late-AHP by α_1-adrenoceptor activation may be mediated by a number of mechanisms. We suggest that augmented release from internal stores is the most likely explanation. The duration of the action potential and the early part of the AHP was not changed by α_1-adrenoceptor activation, suggesting that calcium entry may not be increased. In addition, caffeine, which is known to evoke release from internal stores, prolonged the AHP. Unlike the effect of caffeine, the α_1-adrenoceptor-mediated action involves a calcium store that is ryanodine insensitive. Only thapsigargin, an agent that depletes internal calcium stores non-selectively, altered the augmentation of the late-AHP by activation of α_1-adrenoceptors. By the process of elimination, we speculate that IP_3-sensitive calcium stores mediate this prolongation.

α_1-Adrenoceptor activation has pleiotropic actions

The activation of α_1-adrenoceptors leads to at least two effects on cell excitability. Cells were depolarized through a decrease in potassium conductance and, at the same time, the potassium conductance that mediates the late-AHP was augmented. The second messenger system involved in the α_1-adrenoceptor-induced depolarization remains to be determined, but was distinguished from the prolongation of the late-AHP. The depolarization was not blocked by strong buffering of internal calcium, suggesting that a release and diffusion of calcium to the membrane is not required. The prolongation of the calcium-dependent late-AHP probably results from augmented release of calcium from internal stores and is mediated by activation of phospholipase C. The prolongation of the late-AHP is thapsigargin sensitive but ryanodine insensitive, suggesting that the IP_3-sensitive pool is the source of the calcium involved in this effect. The fact that ryanodine and caffeine also affect the duration of the late-AHP suggests the presence of a second calcium compartment in dorsal raphe neurons. Although the increase in the late-AHP by phenylephrine does not directly affect the ryanodine-sensitive calcium pool, it is possible that the calcium release induced from the IP_3-sensitive pool can mediate calcium-induced calcium release from the ryanodine-sensitive pool (Berridge, 1993). Taken together, the increase in amplitude and duration of the late-AHP limit the excitation caused by the decrease in potassium conductance mediated by

α_1-adrenoceptor activation. Thus the frequency and pattern of firing would be determined by the interplay between the prolongation of the AHP and the depolarization.

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