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Effects of dopamine and noradrenaline on Ca channels of cultured sensory and sympathetic neurons of chick

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Abstract. The effects of noradrenaline and dopamine on voltage-dependent Ca currents were investigated in cultured dorsal root and sympathetic ganglion neurons from chick embryos. At concentrations of 1 to 10 μM, bath application of the neurotransmitters caused a general depression of inward Ca currents. Above −20 mV the decrease of the current amplitude was reversible and accompanied by a 2–10-fold prolongation of the activation time course. Below −20 mV, where a low voltage-activated Ca component is turned on, the size of the currents was reduced by 40% with little effect on the time course. Despite extensive wash-out, little sign of reversibility was observed in this case.

Single-channel current recording in outside-out membrane patches revealed that at low membrane potentials dopamine and noradrenaline reversibly reduced single Ca-channel activity. This finding supports the view that in sensory and sympathetic neurons, both neurotransmitters affect the membrane conductance by modulating Ca permeability and not by activating catecholamine-specific channels able to carry transient outward currents. The probability of Ca channel opening is strongly reduced by addition of 10 μM of either catecholamine to the bath. The possible involvement of a voltage-dependent block of Ca channels by the neurotransmitters is discussed.

Key words: Ca-channels – Noradrenaline – Dopamine – Sensory neurons – Sympathetic neurons – Patch-clamp

Introduction

Dopamine (DA) and noradrenaline (NA) act primarily as inhibitory neurotransmitters on ganglion neurons (Kobayashi and Libet 1970; Christ and Nishi 1971; Dun and Nishi 1974; Krnjević 1974; Zieglgänsberger 1982). However, increasing evidence suggests that catecholamines also interfere with the voltage-sensitive calcium conductance (Dunlap and Fischbach 1978, 1981; Horn and McAfee 1980; Galvan and Adams 1982). As Ca influx is the triggering event of various neuronal activities, including neurotransmitter secretion, it is likely that the catecholamines modulate this function by reducing membrane Ca permeability.

Using the patch-clamp technique (Hamill et al. 1981), we have tested this hypothesis by studying the interaction of catecholamines with the Ca channels in sensory and sympathetic neurons of a vertebrate. Ca current components are prominent in dorsal root (DRG) and sympathetic ganglion (SG) neurons of chick embryos (Dichter and Fischbach 1977; Adams 1981). In particular, chick and rat DRG cells have been shown to possess two types of Ca channels (Carbone and Lux 1984a; Nowycky et al. 1984; Fedulova et al. 1985; Bossu et al. 1985). One, activated at high voltages (HVA), resembles the classical Ca channel of other membrane preparations (for a review see Hagiwara and Byerly 1981). The other, activated at low voltages (LVA), shows fast and complete time dependent inactivation.

Little is known at present about the functional role and drug sensitivity of these two Ca channels. LVA channels have been shown to be implicated in the control of the firing mode of some central neurons (Llinas and Yarom 1981; Llinas and Jahnsen 1982) and to regulate hormone release in endocrine cells (DeRiemer and Sakmann 1986). They are insensitive to high doses of Verapamil (Fedulova et al. 1985; Boll and Lux 1985) and intracellular Ca^{2+} (Bossu et al. 1985; Carbone and Lux 1986a). HVA channels, on the other hand, are sensitive to organic and inorganic Ca antagonists and are implicated in the control of a large number of cellular activities (Hagiwara and Byerly 1981). In this respect we thought it of interest to test the action of DA and NA on the two Ca channels. Our findings suggest that NA and DA affect the two channels differently. They decrease the probability of opening of the LVA type, and produce a strong prolongation of the activation time course of the HVA type.

Some of the present results have already been reported in a preliminary communication (Marchetti et al. 1984).

Materials and methods

Dissociated cell cultures of dorsal root ganglion (DRG) and sympathetic ganglion (SG) neurons from 10 day old chick embryos were prepared according to the method of Barde et al. (1980), and used between 6 h and 4 days after incubation.

The experimental set-up was similar to that already described (Brown et al. 1984; Carbone and Lux 1984a). Patch electrodes were made of Duran glass (Schott Ruhrglas,

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Table 1. External solutions (mM)

Abbreviation	NaCl	$CaCl_2$	$MgCl_2$	Chol-Cl	Glucose	Na-HEPES	pH_o
5 Ca	120	5	2		20	10	7.3
1 Ca	120	1	6		20	10	7.3
120 Chol		5	2	120	20	10	7.3

Internal solutions (mM)

	CsCl	TEACl	$CaCl_2$	EGTA-OH	Glucose	Na-HEPES	pH_i
130 Cs	130	20	0.25	5	10	10	7.3

Mainz, FRG) with a tip resistance of 3–4 MΩ for the whole-cell recording (WCR) and 8–12 MΩ for outside-out patches (OOP). In WCR, currents were measured through a 1 GΩ feedback resistor and filtered at 5 kHz. OOP currents were recorded through a 10 GΩ feedback resistor and low-passed at 1 kHz. Data were stored on an FM magnetic tape, with a bandwidth of 2.5–5 kHz. Analog records were digitized at a frequency of 7–12 kHz by a 12 bit A/D converter and analysed by a LSI 11/23 minicomputer. Leakage and capacitative transients were corrected by subtracting from the membrane current a scaled signal which was the average response of 10 hyperpolarizing pulses of appropriate amplitude. Single channel data were evaluated following the some procedure described by Lux and Brown (1984), and Carbone and Lux (1984b).

All solutions employed are listed in Table 1. The internal medium contained 130 mM Cs and 20 mM TEA to minimize outward potassium currents. Na currents were fully blocked by addition of 3 μM TTX to the bath. We found little or no difference between Ca currents recorded in the presence of 120 mM NaCl and when Na was replaced by choline, suggesting that the contribution of TTX-insensitive Na-currents (Kostyuk et al. 1981a) was negligible in these preparations. Stock solutions of 10^{-2} M DA (3-hydroxytyramine-HCl) and NA(−)arterenolbitartrate (Sigma Chem. Co., St. Louis, MO, USA), were dissolved in bidistilled water containing 1 mg/ml ascorbic acid.

Drug solutions were prepared immediately before the experiment and applied by pressure ejection via a double barreled glass pipette (3–10 μm tip diameter) positioned 50–100 μm from the cell. Control experiments with solutions stained with phenol-red were made to optimize both the position of the ejection pipette and the pressure conditions necessary to achieve complete perfusion of the cell without affecting the electrophysiological response. In OOP measurements, the pipette tip was usually made slightly larger (10 μm) to assure better alignment with the patch at short distance (10–100 μm). Pressure was always switched off before recording.

In WCR, the cell was held at −70 mV, a holding potential which could be easily maintained for long time and which was sufficiently negative to allow the recording of LVA currents (see Carbone and Lux 1984a). Test pulses of 100 ms were applied at intervals of 10 s to minimize possible inactivation of HVA channels. The action of the drug was tested at three or more membrane potentials. Run-down of Ca currents (Kostyuk et al. 1981b; Byerly and Hagiwara 1982; Fenwick et al. 1982) was frequently, but not always observed under these conditions. In some cases, taking the precaution of giving very low frequency stimulations ($1/30\ s^{-1}$), currents persisted for 20–30 min and fully recovered after drug application. A test of current run-down was always done before drug application. Cells showing a rapid loss of Ca currents were abandoned.

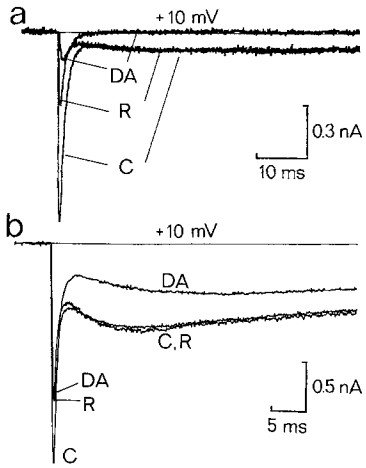

Fig. 1 a, b. Effects of 10 μM DA and Ca inward currents of chick DRG cells at different extracellular Ca concentrations. **a** Time course of inward currents in 1 mM Ca and 120 mM Na (see Table 1) on step depolarization to +10 mV before *C*, during *DA* and after Dopamine application *R*. Note the complete block of the Ca component (persistent inward current) and its full recovery. **b** Simultaneous recording of Na and Ca currents from a cell bathed in 5 mM Ca and 120 mM Na (see Table 1). Block of Ca current by 10 μM DA is incomplete in this case, and the time course of activation is considerably prolonged. Notice that after 5 min of washing the Ca, but not the Na current component, recovers fully. Holding potential was −70 mV in both cases. Internal solution: 130 Cs. Temp. 12° C

Outside-out patches of DRG cells were held at a potential of −90 or −100 mV. Test pulses of 500 ms, up to a membrane potential of −40 mV, were applied at low frequency ($1/30\ s^{-1}$). OOP of SG cells did not last long enough to allow a study of drug application.

Results

Effects of dopamine on Na and Ca currents in DRG cells

In the presence of internal Cs and TEA, inward currents at +10 mV in DRG cells are shown to possess two distinct current components (Carbone and Lux 1986b): an early, fast-inactivating Na component and a late, slowly-inactivating Ca component. As shown in Fig. 1, both currents

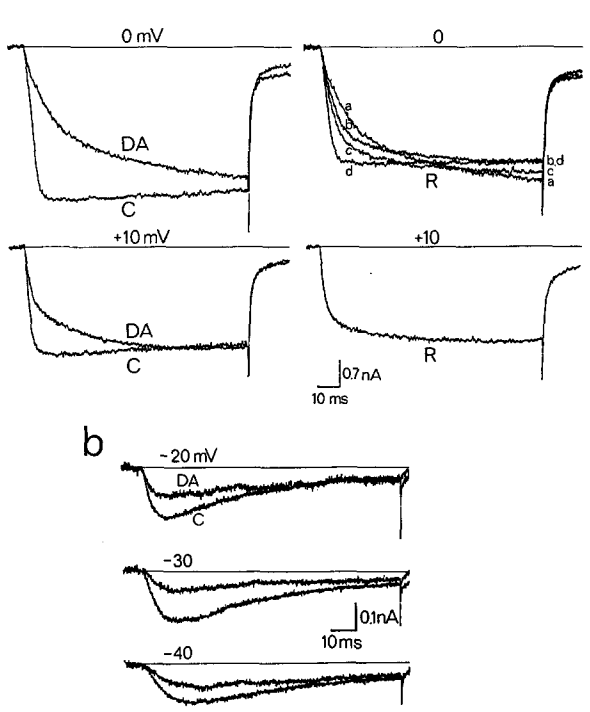

Fig. 2a, b. Effects of 10 μM DA on isolated HVA and LVA Ca currents in chick DRG. **a** HVA currents recorded at 0 and +10 mV, before *C*, during *DA* and after DA application *R*. The right upper part of the figure shows overlapped current traces, recorded at different times during the recovery. *Trace a* was recorded immediately after DA application; *traces b* and *d* after respectively 1, 2 and 5 min of washing. Note the progressive and complete recovery of the activation-inactivation kinetics despite the 20% amplitude reduction of the final record *d*. Out of 33 cells, 27 showed similar effects to those illustrated here, but the recovery for half of them was obscured by current rundown. Six cells failed to exhibit any response to DA. Same conditions as in Fig. 1 except that 3 μM TTX were added to the external bath. Temp. 22° C. **b** LVA currents recorded from the same cell as in **a** at the potentials indicated. In this cell there was no recovery from the action of DA despite 15 min washing (not shown)

were reduced by bath application of 10 μM DA (Fig. 1). Ca currents, however, appeared more depressed than Na currents and the degree of depression decreased with increasing external Ca. DA completely abolished Ca currents in 1 mM Ca (Fig. 1a), and reduced their size only by half in 5 mM Ca (Fig. 1b). In both cases, recovery of Ca currents was nearly complete, while Na currents recovered only partially. This clearly indicates an unspecific action of DA on Ca channels but also suggests somewhat different mechanisms of action of the catecholamine on Na and Ca channels. The full recovery of Ca currents, which are usually more unstable than Na currents (see Methods), speaks against a run-down of the general condition of the cell and reinforces the view that DA acts efficiently and partly irreversibly on the Na channel of chick DRG. This point, however, was not further investigated in this study.

The effects of 10 μM DA on isolated HVA and LVA Ca currents are illustrated in Fig. 2. At 0 mV in the presence of DA, the activation time course of HVA currents was drastically slowed and the current amplitude at the end of a 100 ms pulse was only mildly depressed (Fig. 2a). At +10 mV, there was also almost no reduction of the current amplitude at the end of the pulse, but Ca current activation in 10 μM DA was faster compared to that at lower potentials. As shown on the right hand side of Fig. 2, recovery from DA application was quick and nearly complete. At 0 mV (top-right of Fig. 2a), despite a 20% amplitude reduction, the current trace recorded after 5 min washing (trace d) had nearly the same activation kinetics as the control record shown on the left (trace c). After washing, Ca currents were never observed to reattain the amplitudes of controls in 33 cells tested.

At lower membrane potentials (−40, −20 mV) where LVA channels are activated, application of 10 μM DA produced a 60% reduction of the currents with little effect on their time course (Fig. 2b). In most of the cells tested, the currents at these potentials showed no sign of recovery even after 10−20 min washing. Interestingly, higher concentrations of DA (100 μM) had similar effects on Ca currents (not shown), suggesting that at 10 μM the neurotransmitter had already a saturating action on Ca channels. At lower doses, the action of DA was clearly attenuated but poor reproducibility of the data hampered a reasonable estimate of a dose- response curve.

Analysis of DA action

From the results of Fig. 2 there are indications that block of Ca currents by DA is voltage- and time-dependent. This is best seen in Fig. 3a, where isochronal I−V characteristics in the presence and absence of DA are plotted at $t=10$, 20 and 70 ms. At 10 ms the I−V curve during DA application showed an overall depression and this was strongly attenuated at 70 ms, as if sustained depolarizations to high voltages relieves Ca currents from the initial block of DA. This agrees with the observation that Ca current activation accelerates with increasing voltages (see Figs 2a and 3b) and suggests that, similarly to other drugs (Yeh et al. 1976; Meves and Pichon 1977), the rate of release of DA molecules from their binding site might be regulated by membrane potential (see Discussion). As shown in Fig. 3b, the time course of Ca currents in 10 μM DA can be well fitted with a double exponential, with time constant τ_1 and τ_2 roughly corresponding to the fast and slow component of the overall Ca current. At 0 mV, τ_1 and τ_2 are respectively 3 and 40 times larger than the time constants at control (see legend in Fig. 3b). At +10 mV both time constants are remarkably reduced. τ_1 becomes comparable to that at control (2.43 ms) and τ_2 is reduced to nearly half of that at 0 mV.

Effects of noradrenaline on Ca currents in DRG cells

As shown in Fig. 4, NA affected Ca currents of chick DRG in a similar fashion to DA. At large voltages (0, +10 mV), Ca current activation was prolonged and the current amplitude reduced in the presence of 10 μM NA and 5 mM external Ca (Fig. 4a). However, NA appeared more effective than DA in reducing the steady-state level of Ca currents. At 0 mV, the depression of Ca currents was nearly 70% at the end of the pulse and the rising phase was only slightly prolonged. This agrees with the results of Forscher and Oxford (1985), on the same preparation. These authors found a similar reversible decrease of Ca currents with 10 μM NA. In our case, recovery of Ca currents was not systematically observed in all the cells tested. Out of twelve cells, four showed recovery similar to that shown in Fig. 4a;

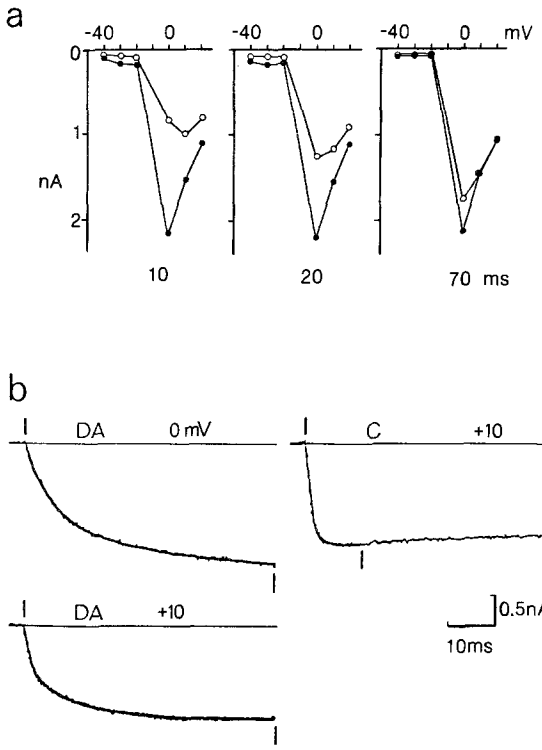

Fig. 3. a Isochronal I–V characteristics calculated at $t=10$, 20 and 70 ms from the onset of depolarization for the current records of Fig. 2. *Filled circles* refer to control Ca currents, *open circles* to currents in the presence of DA. **b** Computer simulation of the time course of Ca currents at control *C* and during *DA* application at 0 and +10 mV. The current traces are from Fig. 2a. *Dotted curves* drawn on top of current records are best fitting functions of the type: $-I(t) = A[1-\exp(-t/\tau_1)] + B[1-\exp(-t/\tau_2)]$ generated for trace DA at 0 mV with $A = 1.03$ and $B = 1.05$ nA, $\tau_1 = 9.03$ and $\tau_2 = 42.1$ ms. The values for the DA trace at +10 mV were $A = 0.7$, $B = 0.86$ nA, $\tau_1 = 2.2$, $\tau_2 = 18.1$ ms. The rise to peak of the control *C* at +10 mV is displayed with $A = 1.7$ nA, $\tau_1 = 2.43$ ms and $B = 0$. Except for the early part of the rising phase of current records, theoretical and experimental data show an overall good overlapping. To account for the early sigmoidal rise of Ca currents a third exponential term would have been required. It was omitted since it did not essentially improve the determination of the time constant of the slow exponential term, τ_2, that is the parameter of main interest of our analysis. *Upper* and *lower bars* on each record indicate the time limits used for the fitting

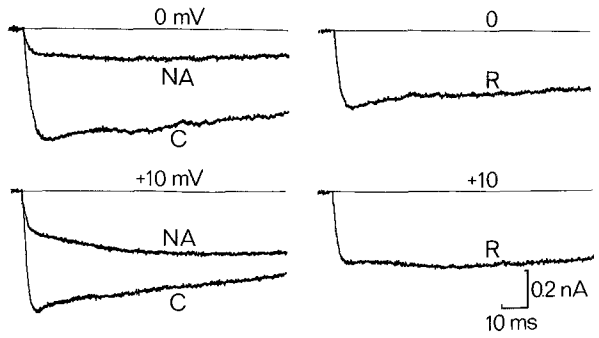

Fig. 4. Effects of 10 μM *NA* on *Ca* currents of chick DRG. Records are taken from the same cell on step depolarizations to 0 and +10 mV. The external solution contained 5 mM Ca, 120 mM choline and 3 μM TTX. V_h: −70 mV. In: 130 Cs. Temp. 22° C

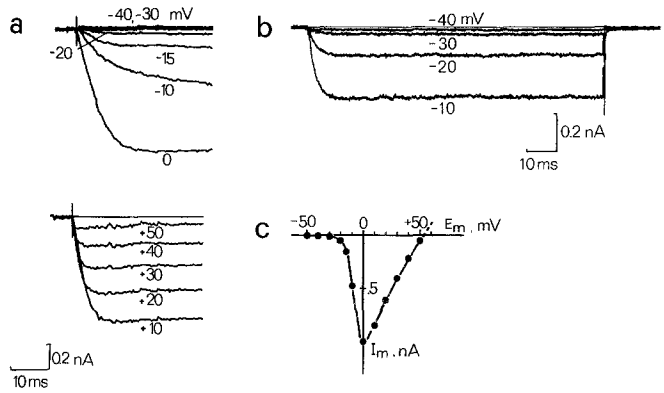

Fig. 5 a–c. Ca currents in chick sympathetic neurons. **a** Time course of Ca currents in 5 mM Ca recorded at the potential indicated. V_h: −80 mV. Out: 5 Ca, 3 μM TTX. In: 130 Cs. Temp. 22°C. **b** Time course of Ca currents at low membrane potentials (−40 to −10 mV) from a holding potential of −70 mV. Repolarizations to −70 mV. Notice that at −40 and −30 mV, where LVA currents normally activate (see Fig. 2b), Ca currents are still small and do not show any sign of time-dependent inactivation during steps lasting 100 ms. **c** I–V relationship relative to the current records of part **a**

four were similarly affected but the currents did not recover after approximately 20 min washing, and four exhibited a non-specific rundown. As with DA, the action of NA was found to be sensitive to external Ca in one cell, but this point was not investigated in more detail.

Action of DA and NA on Ca currents in SG cells

As shown in Fig. 5a, Ca currents in chick sympathetic neurons had strong similarities to those of other previously described preparations (Adams 1981; Fenwick et al. 1982; Hagiwara and Ohmori 1982). Currents start to be activated at −30 mV, reach maximal amplitude between 0 and +10 mV, and reverse near +55 mV. They turn on rapidly between −30 and +50 mV and show almost no sign of inactivation during pulses of 100 ms duration. Several lines of evidence indicate that these cells do not possess LVA channels as observed in chick and rat DRG (Carbone and Lux 1984a; Fedulova et al. 1985; Bossu et al. 1985) : (i) the I–V relationship shows only one inflection point at approximately −10 mV (Fig. 5c), (ii) at low membrane potentials (−40 to −20 mV), there is no sign of time-dependent inactivation (Fig. 5b), and (iii) closing of channels is very fast (<1 ms), as inferred from the time course of tail currents on repolarization to −70 mV (Fig. 5b).

Consequently, sympathetic neurons were chosen as a model system to study the action of DA and NA on a cell membrane containing an homogeneous population of HVA channels. Figure 6a shows that the effects of 10 μM DA (or NA) on the Ca currents of SG cells are not substantially different from those of DRG. At +10 mV, Ca current activation is considerably prolonged and the steady-state level of the current is only mildly depressed. This rules out the possibility that LVA channels are implicated in the slowing down of Ca current activation at high voltages and

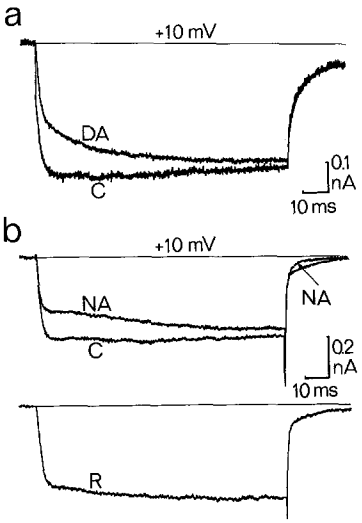

Fig. 6. a Effects of 10 μM DA on Ca currents in SG neurons. Step depolarizations were to +10 mV from an holding potential of −70 mV. The external bath contained 5 mM Ca, 120 mM choline and 3 μM TTX. Temp. 22° C. **b** Effects of 10 μM NA on Ca currents. Same conditions as in part **a** except that choline was replaced with Na. V_h: −70 mV

suggests specific interaction of the two catecholamines with the activation rates of HVA channels.

Action of DA and NA on single Ca currents in outside-out patches

OOP recordings in DRG cells were made under ionic conditions similar to those used in WCR. At control in 5 mM Ca, single Ca channel activity could be observed on step depolarizations to −40 mV from holding potentials of −90 and −100 mV (Fig. 7a). Amplitude and mean open time distributions of single current events suggested a predominant activity of the LVA channel (Carbone and Lux 1984b). Application of 10 μM DA (or NA) markedly suppressed the occurrence of channel opening without much effect on single channel conductance (Fig. 7b). Also, the mean open time showed a slight decrease in the presence of the catecholamines in several OOP's. However, the available data, yielded no specific kinetic information on the mechanism of action of the catecholamines. In the potential range analysed (−40, 0 mV), application of DA to OOP never produced discrete events of outward currents. This ruled out the possibility that the observed reduction of Ca conductance by DA was due to the activation of catecholamine-specific channels able to carry transient outward currents. No attempt was made to investigate the effects of DA on cell-attached patches

On sample averages, the action of DA (or NA) resulted in a reversible depression of the peak current amplitude (Fig. 8). Recovery of channel activity was nearly 70% for DA and 50% for NA. This contrast with the experiments on WCR at low potentials (Fig. 2b), in which no recovery of channel activity was observed after about 20 min of continuous washing. This suggests that the persistence of DA action is very likely mediated by processes which depend on the intact cell interior.

Interestingly, in four OOP formed in solutions containing 20 mM Ca, neither DA nor NA had a clear effect on Ca channels. This is in line with our previous observation that the action of the catecholamines is reduced by increasing the external Ca concentration. This point, however, was not further investigated in the present work. Also, no attempts have been made to ascertain whether the antagonist action of Ca on catecholamines was specific for Ca ions or was a general property of divalent cations (Ba^{2+}, Sr^{2+} and Mg^{2+}).

Discussion

Our results show that catecholamines affect differently the HVA and LVA Ca currents of sensory and sympathetic neurons. Bath applications of 10 μM DA reversibly prolonged the activation kinetics of HVA currents and reduced the size of LVA currents with little changes in their activation-inactivation kinetics. In this respect, both catecholamines provide an additional entry in the list of substances which show different sensitivity for the two Ca channels (Nowycky et al. 1984; Fedulova et al. 1985; Boll and Lux 1985; Carbone and Lux 1986a; Bossu et al. 1985).

From the present data little can be inferred about the mechanism of action of DA and NA on the two Ca channels. One possibility is that the two catecholamines act separately on the two channels: modifying reversibly the activation gates of the HVA type and causing irreversible alterations to the channel gating mechanism of the LVA type. In this case the action of catecholamines would be indicative of the presence of specific receptors at the two channels, but our data do not allow any direct identification with other receptors such as those previously described for opioids (for a review see Creese et al. 1983).

A second possibility is that DA and NA affect HVA and LVA channels in a similar way and that the dissociation constant of the drug-channel complex is controlled by membrane potential. Under this assumption, DA and NA would be expected to bind to Ca channels preferentially at negative potentials and to dissociate with rates which would increase with increasing membrane depolarizations (see Yeh et al. 1976; Meves and Pichon 1977 for a similar model). If the rate of drug removal is much smaller than the rate of channel gating such a mechanism would produce a prolongation of Ca activation at large potentials and little distortion to the time course of Ca currents at weak depolarizations (see Fig. 2). Under this assumption the slow phase of Ca activation observed in Figs. 2 and 3b (τ_2 = 42.1 at 0 mV and τ_2 = 18.1 ms at +10 mV) can be taken as a direct estimate of the time constant of drug removal at these potentials.

An interesting prediction of this model is that with repetitive depolarizations at large potentials (0; +10 mV), Ca currents would show a gradual recovery from DA-blockade with time. Ca activation, which is slow during single depolarizing steps in 10 μM DA, would accelerate after successive depolarizations. We have made several attempts to verify this point, but in all cases the fast run-down of Ca currents occurring at high rates of stimulations (1−3 per second) did not allow us to draw any definite conclusions from our experiments.

The effects of NA on Ca currents shown in Figs. 4 and 6 agree partially with previous observations on chick sensory neurons (Dunlap and Fischbach 1981; Forscher and Oxford 1985) and rat sympathetic ganglions (Galvan and Adams 1982). In both cases, application of 10 to 100 μM NA caused

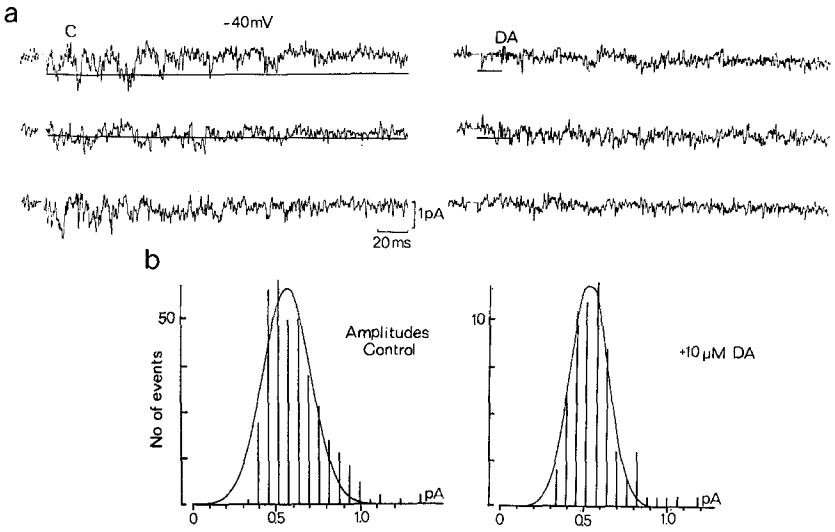

Fig. 7 a, b
Action of 10 μM DA on single Ca channels in OOP of chick DRG. **a** Current traces recorded before (*left*) and after 2 min application of DA (*right*). The lines in the current traces indicate the detection levels chosen for channel opening (*top trace*) and closing (*middle trace.*) Leakage and capacitive currents were compensated as described in Methods. A blank of 3 to 5 ms was introduced at the onset of the current trace to cancel residual artifacts. Records were low-passed at 900 Hz. V_h: −90 mV. Out: 5 Ca. Temp. 22° C. **b** Amplitude histograms for 12 traces at control (294 events) and during application of DA (60 events)

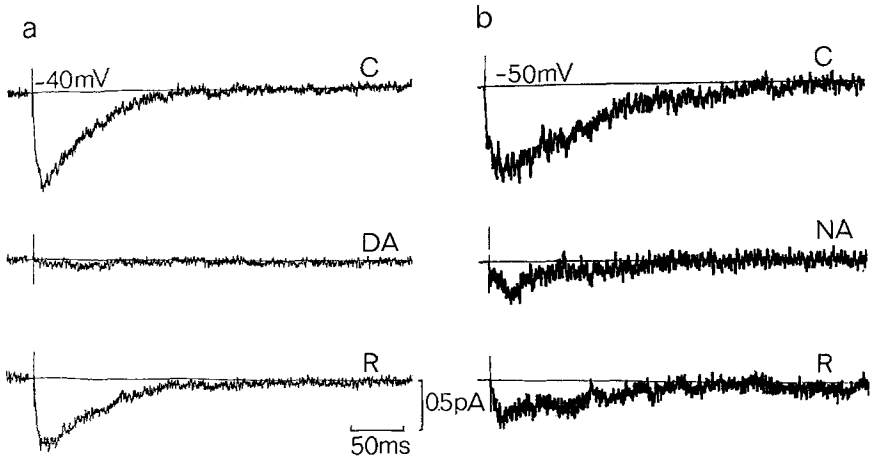

Fig. 8 a, b
Effects of 10 μM DA (*left*) and NA (*right*) on current sample averages from OOP of chick DRG. **a** Averages of 10 single channel recordings, before *C* during *DA* and after application of dopamine *R*. Notice that at control the current records are strongly inactivated. This is due to the predominant activity of the LVA channel which is know to survive for longer times in OOP than the HVA type (Carbone and Lux 1985). The patch apparently contains a large number of channels (5–10) whose value, however, could not be exactly estimated. Conditions were similar to Fig. 7. Traces were filtered at 500 Hz. **b** Averages of 8 single channel traces. V_h: −90 mV. The cut-off frequency was 1 kHz. Out: 120 choline. 3 μM TTX

a depression of HVA currents with little change in the activation kinetics. This contrasts with our findings, but it can be partly explained if block of HVA channels is assumed to be voltage-dependent. According to this hypothesis, low holding potentials (−40 to −60 mV) and short or small step depolarizations, as previously used, would attenuate considerably the block release of NA from its binding site with consequent reduction of the slow phase of Ca current activation. This holds true also for the action of other related substances, such as 5-HT (Dunlap and Fischbach 1981), GABA (Deisz and Lux 1985) and glycine (D. Sakmann, personal communication) which have been shown to have similar effects on Ca currents. To our knowledge, there are no detailed reports on the effects of DA on Ca currents which can be compared with our present findings.

Although not fully specific, the observed effects of DA and NA on Ca currents are of pharmacological relevance. The drastic slowing down of HVA currents occurs on a time scale which is much longer than that of the Ca-dependent spike at the soma (2–10 ms). This implies that the depression of 60% of HVA currents in the first 10 ms of a pulse (Fig. 3a) would be sufficient to produce a drastic reduction of the action potential duration as previously reported in cultured sensory neurons (Dunlap and Fischbach 1978; Mudge et al. 1979; Werz and Macdonald 1983). In this respect, even lower doses of the drugs are likely to be highly effective. In particular, the action of the two catecholamines might be more effective at physiological conditions where the extracellular Ca concentration is lower (1–2 mM) than that used in our studies. Since low $[Ca]_o$ enhances the blocking action of DA and NA (Fig. 1), it is possible that much lower doses of the two drugs (0.1–1 μM) are sufficient to produce a significant shortening of the soma spike in sensory neurons.

The depression of LVA currents caused by catecholamines could be relevant for the functioning of certain neurons. LVA Ca currents have been proposed to control the oscillatory behaviour of several central neurons by inducing secondary Na-Ca spikes evoked by a triggering spike after sufficiently long repolarizations (Llinas and Yarom 1981). If this is the case, a 60% depression of Ca currents (see Fig. 2) would be expected to produce a considerable slow-down of the depolarizing phase which precedes the generation of a secondary spike (see Fig. 7 in Llinas and Yarom 1981), with a consequent change of the firing threshold and burst-frequency of the neuron. This might be the reason why bath applications of 0.1–10 μM DA have been observed to produce threshold spike elevations in CA1

hippocampal neurons (Stanzione et al. 1984) and to inhibit burst-firing in neurosecretory cells of Aplysia (Gaspe and Wilson 1984).

At the presynaptic level, Ca channel block as described here would have drastic effects. Decreases of Ca entry could inhibit the release of excitatory neurotransmitters and block EPSP's. Another putative neurotransmitter, the opioid peptide enkephalin (Konishi et al. 1981) has been shown to have such an action: it decreases action potential duration in sensory neurons, probably by reducing the Ca-dependent spike component (Mudge et al. 1979), and reduces quantal content at the frog neuromuscular junctions (Bixby and Spitzer 1983).

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