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CHARACTERISTICS OF THE ELECTRICAL RESPONSE TO DOPAMINE IN NEUROBLASTOMA CELLS

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SUMMARY

1. The characteristics of the electrical response to dopamine in the mouse neuroblastoma cell line N1E-115 were studied.

2. Neuroblastoma cells responded to ionophoretically applied dopamine by generating a transient depolarization. Under voltage-clamp conditions, a transient inward current was recorded in response to dopamine application.

3. The receptor was more effectively activated by dopamine than by noradrenaline. Haloperidol blocked the dopamine-induced current with an apparent dissociation constant of 40 nM. Phentolamine was much less potent than haloperidol, and propranolol had no effect.

4. The dopamine-induced current was increased in amplitude by hyperpolarizing the membrane, decreased by depolarization, and reversed its polarity at +14 mV.

5. When the external sodium concentration was decreased from 125 to 94 mM, the reversal potential was shifted in the direction of hyperpolarization by 10 mV.

6. Increasing the external potassium concentration from 0·2 to 20 mM caused a shift of the reversal potential by 13 mV in the direction of depolarization.

7. Replacement of external chloride with isethionate or glutamate caused little or no shift in the reversal potential, but increased the amplitude of the current.

8. Increase in external calcium concentration caused a block of the dopamine-induced current with an apparent dissociation constant of 1·3 mM, without altering its reversal potential.

9. It is concluded that the ionic channel activated by dopamine undergoes a conductance increase to both sodium and potassium but not to chloride or calcium.

INTRODUCTION

Dopamine is not only a precursor of noradrenaline but also a putative neurotransmitter in the central and peripheral nervous systems. There are various lines of evidence that dopamine is a neurotransmitter in both invertebrate and vertebrate synapses (Commissiong & Neff, 1979; Creese, Sibley, Leff & Hamblin, 1981; Krnjevic,

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1974). However, the response induced by dopamine through the post-synaptic receptor has not been well characterized yet.

Neuroblastoma cells are a highly suitable preparation for the study of dopamine-induced responses for several reasons. First, they are clones derived from mammalian lines and possess various types of receptors responsive to neurotransmitters thereby serving as an excellent model for the central nervous system. Secondly, the presence of a variety of catecholamine systems has been demonstrated in neuroblastoma cells. Dopamine, noradrenaline, adrenaline and serotonin were found in these cells (Mandel, Ciesielski-Treska, Hermetet, Zwiller, Mack & Goridis, 1973*a*, *b*; Narotzky & Bondareff, 1974; Breakefield, 1975). Uptake of L-tyrosine (Richelson, 1974; Richelson & Thompson, 1973) and dopamine (Breakefield, 1975) was demonstrated. Dopamine synthesis and various enzymes were discovered including tyrosine hydroxylase activity (Amano, Richelson & Nirenberg, 1972; Richelson, 1973, 1976; Waymire, Weiner & Prasad, 1972), dopamine β-hydroxylase activity (De Potter, Fraeyman, Palm & DeSchaepdryver, 1978; Mandel *et al.* 1973*b*), catechol-O-methyl transferase activity (Skaper, Adelson & Seegmiller, 1976), and monoamine oxidase activity (Skaper *et al.* 1976; Donnelly, Richelson & Murphy, 1976; Hawkins & Breakefield, 1978). Thirdly, dopamine has been found to cause depolarizing and/or hyperpolarizing responses in neuroblastoma cell lines (Christian, Nelson, Bullock, Mullinax & Nirenberg, 1978; Myers & Livengood, 1975; Myers, Livengood & Shain, 1977; Peacock & Nelson, 1973; Traber, Reiser, Fischer & Hamprecht, 1975). Fourthly, a variety of advanced electrophysiological techniques is applicable to neuroblastoma cells under visual control *in vitro*.

The electrophysiological studies quoted above were conducted under current-clamp conditions which did not allow precise measurements of various membrane parameters to be made. In order to characterize the dopamine response, voltage-clamp experiments have been performed using the mouse neuroblastoma cell line N1E-115. We found that the transient depolarization in response to ionophoretic application of dopamine is due to a transient inward current which is produced by an increase in membrane conductance to both sodium and potassium, but not to chloride or calcium.

METHODS

Neuroblastoma cells of the N1E-115 line were plated in plastic tissue culture dishes containing Dulbecco's Modified Eagle Medium with 10% fetal calf serum added. The dishes were maintained in a humidified atmosphere of 10% CO_2 and 90% O_2 at 37 °C. N^6,O^{12}-dibutyryl adenosine 3':5'-cyclic monophosphoric acid (dibutyryl cyclic AMP) was added to cultures at a concentration of 1 mM in order to enhance the responses to dopamine. Cells were exposed to dibutyryl cyclic AMP for 3 d to 2 weeks before being used for the experiments.

Glass micro-electrodes filled with 3 M-KCl having a tip resistance of 10–50 MΩ were used for recording membrane potential. Dopamine was applied to the cell by ionophoresis through an extracellular micropipette filled with 1 M-dopamine solution. The tip resistance of these micropipettes varied from 10 to 50 MΩ. The frequency of ionophoretic dopamine applications was kept low at 0·02 Hz in order to allow recovery from desensitization.

For voltage-clamp experiments, a second micro-electrode filled with 3 M-KCl having a resistance of 30 MΩ was inserted into the cell, and used for passing current. A standard two micro-electrode voltage-clamp technique was utilized. Membrane current was measured with a current-to-voltage converter which held the bath at the virtual ground. Cell input resistance was also measured by applying constant current pulses across the membrane with the second intracellular micro-electrode.

The saline solution used had the following composition (mM): NaCl, 125; KCl, 5·5: $CaCl_2$, 1·8;

$MgCl_2$, 0·8; HEPES, 20; dextrose, 25; and sucrose, 36·5. The pH of the solution was adjusted to 7·3 with 1 M-NaOH. The osmolarities of the normal saline solution and those with altered cation composition were adjusted to 340 mOSM by the addition of sucrose. A chloride-deficient solution was prepared by replacing NaCl with Na isethionate or Na glutamate. All experiments were carried out at a temperature of 30 to 35 °C.

3-Hydroxytryamine (dopamine), noradrenaline HCl, propranolol HCl and N^6,O^{12}-dibutyryl-adenosine 3′:5′-cyclic monophosphoric acid were obtained from Sigma Chemical Co., St. Louis, MO. Phentolamine was obtained from Ciba Pharmaceutical Co., Summit, NJ. Haloperidol was a gift from McNeil Laboratories, Fort Washington, PA.

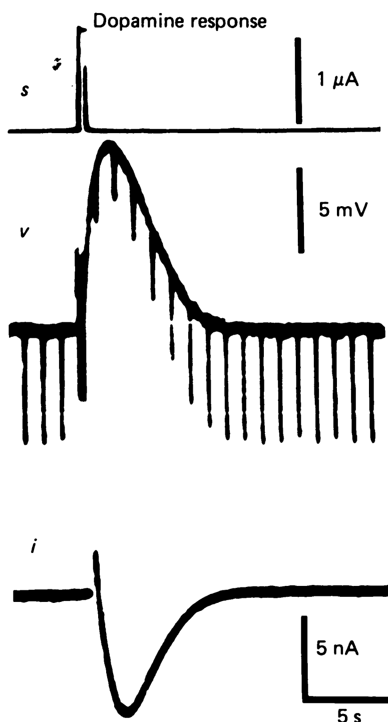

Fig. 1. A transient depolarizing response and inward current induced by ionophoretic application of dopamine. *S*, *V* and *I* indicate, respectively, ionophoretic stimulating current, the dopamine-induced potential which is accompanied by a decrease in the response to brief hyperpolarizing constant current pulses applied to the membrane, and the dopamine-induced current recorded by voltage-clamp method. Both records were taken from the same cell at a membrane potential of −30 mV.

RESULTS

Response induced by ionophoretic application of dopamine

When dopamine was applied ionophoretically to a neuroblastoma cell, a transient depolarizing response was observed under current-clamp conditions. This depolarization was accompanied by an increase in membrane conductance as can be seen from a decrease in amplitude of hyperpolarizations in response to constant current pulses applied to the membrane through a second intracellular micro-electrode (Fig. 1,

middle record). The intensity of ionophoretic stimulation necessary for evoking measurable responses ranged from 10 to 500 nC. The sensitivity to dopamine was very low in some cells.

The bottom record of Fig. 1 shows an inward membrane current under voltage-clamp conditions as induced by an ionophoretic application of dopamine. The mean amplitude, the time to peak amplitude and the half-decay time of the dopamine-induced current for the stimulus intensity used were estimated to be $6 \pm 0{\cdot}7$ nA, $1{\cdot}1 \pm 0{\cdot}1$ s and $1{\cdot}8 \pm 0{\cdot}1$ s ($n = 18$), respectively. The transient depolarizing response was sometimes followed by a small hyperpolarizing response. In these rare cases, an inward current was followed by an outward current under voltage-clamp conditions.

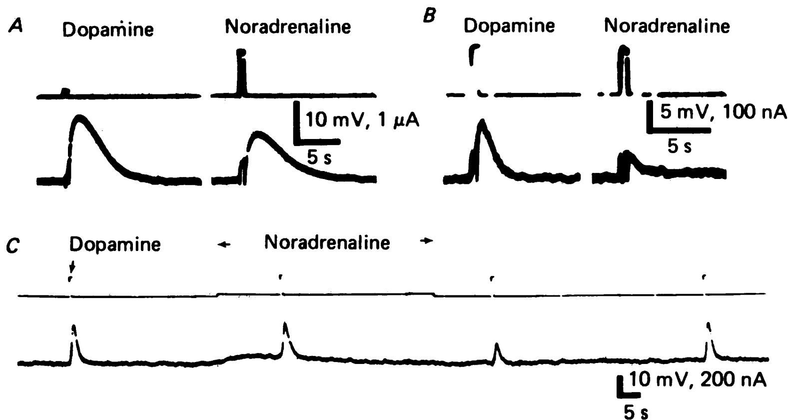

Fig. 2. *A*, dopamine- and noradrenaline-induced depolarizing responses. The dopamine ionophoretic stimulation was smaller than the noradrenaline stimulation. Both records were taken from the same cell. *B*, dopamine and noradrenaline responses induced by the same ionophoretic stimulus intensity. Both records were taken from the same cell. *C*, effect of a prolonged ionophoresis of noradrenaline on the dopamine potential. The resting membrane potentials of the cells used for records *A*, *B* and *C* were −40, −50 and −55 mV, respectively.

Comparison of dopamine and noradrenaline

In order to determine the specificity of the receptor, dopamine and noradrenaline were compared for their ability to induce responses. Two ionophoretic micropipettes, one containing dopamine and the other noradrenaline, were positioned very close to each other on a single cell. Fig. 2*A* is an example of a pair of records in which noradrenaline induced a depolarizing response smaller than that induced by dopamine even though a much stronger ionophoretic current was applied to the noradrenaline micropipette. The smaller efficacy of noradrenaline is more clearly seen in Fig. 2*B* in which a stimulus of the same intensity induced a much smaller response by noradrenaline than by dopamine. This difference may be at least in part due to the possible difference in transport numbers for dopamine and noradrenaline.

Noradrenaline desensitized the membrane to dopamine, and dopamine desensitized

the membrane to noradrenaline. Fig. 2*C* shows that the dopamine-induced potential was diminished by a prolonged ionophoretic application of noradrenaline. This cross desensitization suggests that dopamine and noradrenaline act on a common receptor. The desensitization of the dopamine response was further characterized by the experiments shown in Fig. 3. Dopamine-induced inward currents were measured

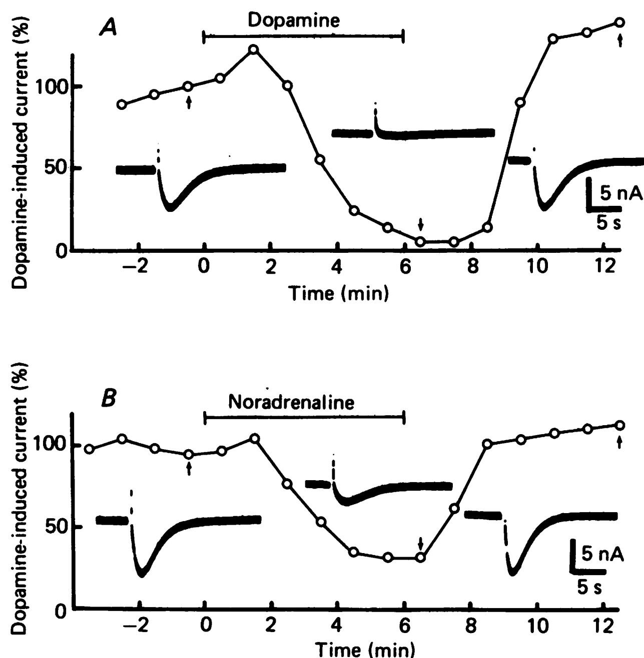

Fig. 3. Effects of bath-application of 100 μM-dopamine (*A*) and 100 μM-noradrenaline (*B*) on the transient inward current induced by ionophoretic application of dopamine. The ordinate represents the peak amplitude of dopamine-induced current as the percentage of the control. The inset shows the records of the dopamine-induced current taken before, during and after bath application of dopamine or noradrenaline at the times indicated by arrows. Experiments *A* and *B* were conducted with the same cell, with *B* being done 10 min after the end of *A*.

under voltage-clamp conditions to avoid complications due to membrane potential changes while dopamine or noradrenaline was added to the bathing medium. In Fig. 3*A*, the ionophoretically induced dopamine current gradually diminished and eventually disappeared during bath-application of dopamine at a concentration of 100 μM. The response reappeared after dopamine was removed from the bath. Fig. 3*B* illustrates a similar experiment in which noradrenaline was added to the bath at the same concentration. The decrease in the dopamine-induced current was less pronounced than that caused by the equimolar concentration of dopamine in the bath. Similar results were obtained in all of four cells tested. Assuming that receptor desensitization varies with receptor activation, this result suggests a higher affinity of the receptor to dopamine than noradrenaline.

Effects of adrenergic antagonists

In order to further characterize the dopamine-induced response, the effects of three antagonists known to act on adrenergic and dopaminergic receptors were studied. Dopamine-induced currents were measured in the presence of antagonists at various concentrations. Fig. 4 depicts dose–response relationships for the action of these drugs. The dopaminergic antagonist haloperidol decreased dopamine-induced current with an apparent dissociation constant of 40 nM. The current was decreased to

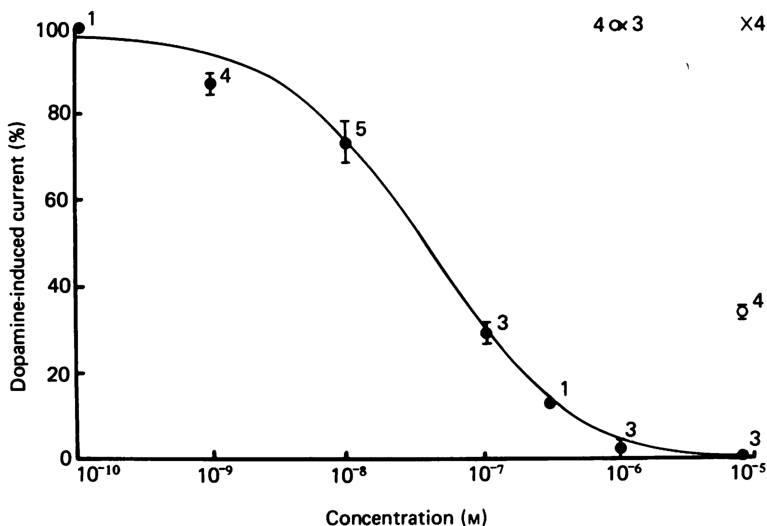

Fig. 4. Dose–response relationships for the actions of adrenergic antagonists haloperidol, phentalamine and propranolol on the peak dopamine-induced current. Each measurement represents the mean ± S.E. of mean with the number of experiments. ●, haloperidol; ○, phentolamine; X, propanolol.

72·6 ± 5·1 % ($n = 5$) of the control at a concentration of 10 nM and blocked completely at 1–8 μM. Phentolamine, an α-adrenergic antagonist, had no effect on the dopamine-induced current at a concentration of 1 μM, but decreased it to 29·3 ± 1·3 % ($n = 4$) of the control at 8 μM. Propranolol, a β-adrenergic antagonist, had no effect on the dopamine-induced current at a concentration of 1–8 μM. The results together with those in foregoing sections lead to the conclusion that we are here dealing with a dopamine receptor.

Ionic mechanism of dopamine-induced response

The ionic mechanism of the dopamine-induced response was studied under voltage-clamp conditions. A family of dopamine-induced currents at various membrane potentials is illustrated in Fig. 5*A*. The membrane potential was held at the resting level (−20 mV), and step hyperpolarizing pulses (left-hand column) or step depolarizing pulses (right-hand column) lasting for 12 s were applied. Dopamine was applied ionophoretically 2 s after the beginning of the pulse. The initial step inward currents during hyperpolarizing pulses and the initial step outward current during

depolarization to −4 mV represent leakage currents. The large outward transient currents associated with longer depolarizations are potassium currents (Moolenaar & Spector, 1978; Quandt & Narahashi, 1979). The currents induced by ionophoretic applications of dopamine flowed inward at large negative membrane potentials and

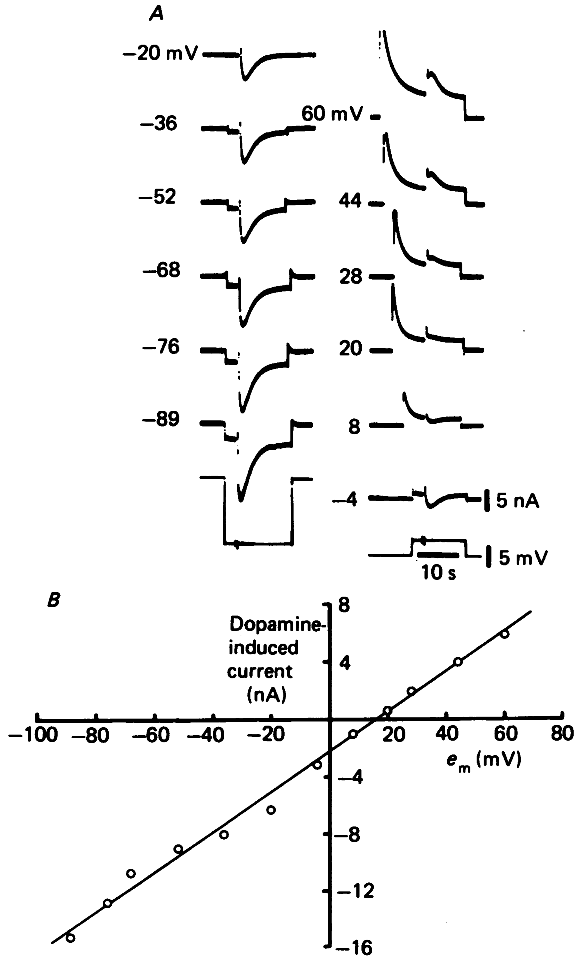

Fig. 5. *A*, dopamine-induced currents evoked at various levels of membrane potential indicated at the left-hand side of each record. Square pulses at the bottom represent samples of clamped hyperpolarizing and depolaring membrane potentials. The dopamine-induced currents are superimposed on the current associated with the membrane potential change. *B*, relationship between the amplitude of the peak dopamine-induced current (ordinate) and membrane potential (abscissa). Negative currents indicate inward currents.

outward at positive membrane potentials. The peak amplitudes of dopamine-induced currents are plotted as a function of the membrane potential in Fig. 5*B*. In this case the current–voltage relationship was linear over the membrane potential range used (−89 mV to +60 mV) with a reversal potential at +17 mV. The mean reversal potential was estimated to be $+13{\cdot}8 \pm 0{\cdot}8$ mV (S.E. of mean, $n = 32$).

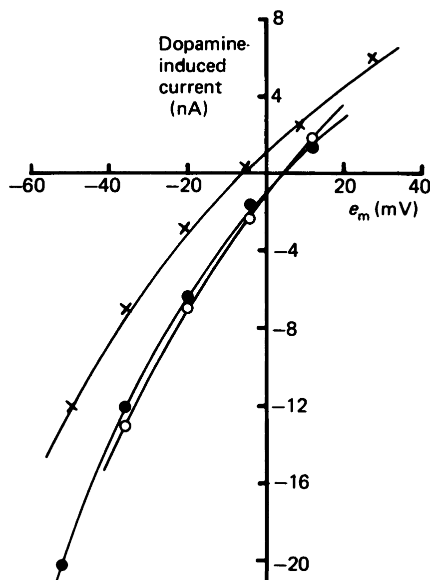

Fig. 6. Effect of a decrease in external sodium concentration from 125 to 94 mM on the relationship between the peak dopamine-induced current and membrane potential. O, 125 mM-Na (control); X, 94 mM-Na; ●, 125 mM-Na (wash).

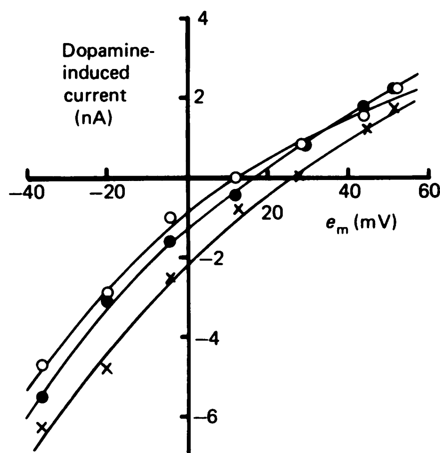

Fig. 7. Effect of an increase in external potassium concentration from 0·2 mM to 20 mM on the relationship between the peak dopamine-induced current and membrane potential. O, 0·2 mM-K (control); X, 20 mM-K; ●, 0·2 mM-K (wash).

In order to determine what ion or ions were involved in the dopamine-induced current, the reversal potential was measured in various concentrations of sodium, potassium, chloride and calcium in the external medium.

Sodium ions. When the external NaCl concentration was decreased from the normal value of 125 mM to 94 mM, the reversal potential for the dopamine-induced current was shifted in the direction of hyperpolarization (Fig. 6). In four experiments, the

shift was estimated to be 10·5 ± 1·3 mV (S.E. of mean). The effect was reversible after washing with normal solution (Fig. 6). It will be shown later that chloride concentration has little or no effect on the reversal potential. Furthermore, the potassium and calcium concentrations in the bathing solution were kept constant. Therefore, the shift of reversal potential should be attributed to the change in the concentration

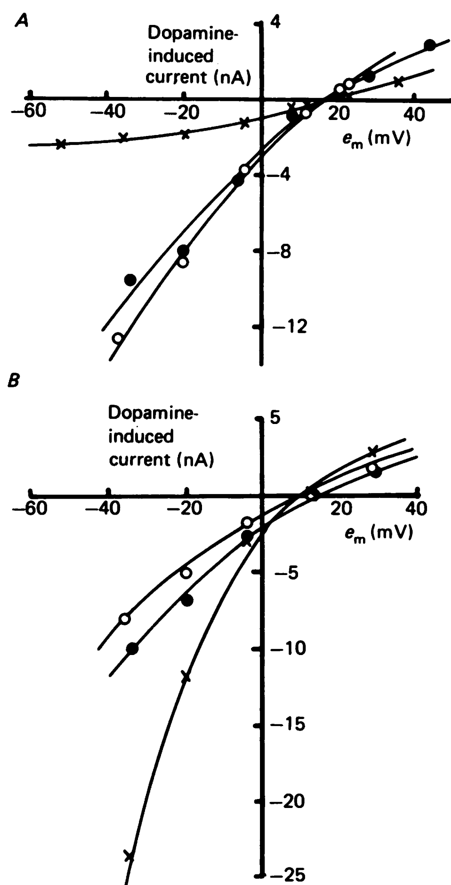

Fig. 8. Effects of an increase in external calcium concentration from 1·8 to 10 mM (*A*) and of a decrease from 1·8 to 0·18 mM (*B*) on the relationship between the peak dopamine-induced current and membrane potential. *A*: ○, 1·8 mM-Ca (control); X, 10 mM-Ca; ●, 1·8 mM-Ca (wash). *B*: ○, 1·8 mM-Ca (control); X, 0·18 mM-Ca; ●, 1·8 mM-Ca (wash).

of sodium. This result indicates that the channel increases its conductance to sodium during the action of dopamine.

Potassium ions. Fig. 7 shows that increasing the external potassium concentration from 0·2 to 20 mM causes a shift of the reversal potential in the direction of depolarization. The mean shift is estimated to be 13 ± 0·6 mV ($n = 3$). The effect was reversible after washing with the control solution containing 0·2 mM-potassium. This indicates that the channel conductance to potassium also increases during the action of dopamine.

Chloride ions. In order to determine whether chloride plays a role in the production of dopamine response, chloride was replaced with isethionate or glutamate, either of which appears to be too large to pass through the channel. Replacement of external chloride with isethionate or glutamate caused little or no shift in the reversal potential, but increased the amplitude of the current. This indicates that the ionic channel associated with the dopamine receptor does not undergo an increase in conductance to chloride during the action of dopamine.

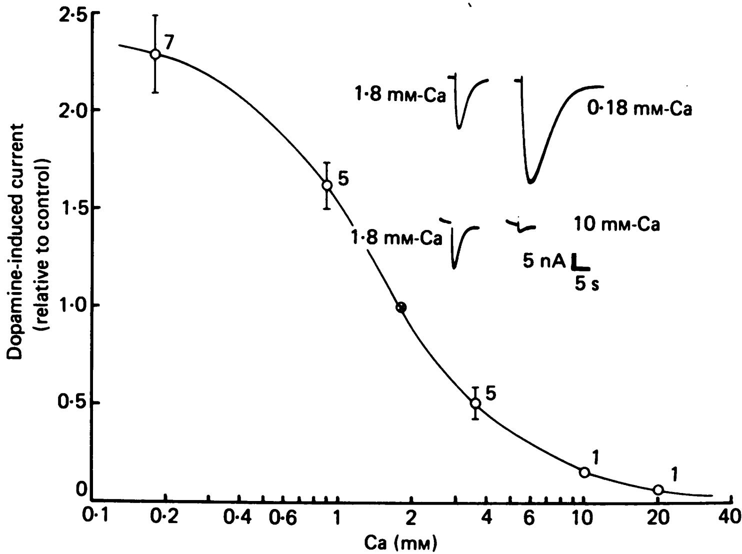

Fig. 9. Effects of external calcium concentration on the peak amplitude of the dopamine-induced current recorded at the membrane potential of −36 mV. Ordinate represents the current amplitude relative to the control at 1·8 mM-calcium. Each measurement represents the mean and S.E. of mean with the number of measurements beside. Inset: upper records show the effect of low Ca (0·18 mM) on the dopamine-induced current, and lower records the effect of high Ca (10 mM).

Calcium ions. The reversal potential for the dopamine-induced current remained the same when the external calcium concentration was increased from 1·8 to 10 mM or decreased from 1·8 to 0·18 mM (Fig. 8). This result indicates that calcium permeability does not increase during the activation of the receptor by dopamine. However, alterations of calcium concentration changed the amplitude of the dopamine-induced current which decreased when the calcium concentration was increased. The dose–response relationship for the Ca-induced decrease in dopamine current is illustrated in Fig. 9. The ordinate represents the amplitude of current relative to the control in normal calcium concentration of 1·8 mM. The inset shows examples of the dopamine-induced current in various calcium solutions. All currents were recorded at −36 mV. An apparent dissociation constant is estimated to be 1·3 mM. These results, together with those in the foregoing sections, lead to the conclusion that the ionic channel activated by dopamine undergoes a conductance increase to both

sodium and potassium, but not to chloride or calcium. However, calcium modulates the dopamine response by reducing the conductance increase in response to dopamine with an apparent dissociation constant of 1·3 mM.

DISCUSSION

The mouse neuroblastoma cell line N1E-115 possesses a receptor which can be activated by the ionophoretic application of dopamine. The cell responds to dopamine with a transient depolarization which is due to a transient inward current as revealed by voltage-clamp experiments. In order to obtain the dopamine-induced current of 6 nA, 10–500 nC of ionophoretic stimulation is necessary. The hybrid cell NG108-15 (Myers *et al.* 1977) has approximately the same dopamine sensitivity as N1E-115. Two kinds of dopamine responses were reported in other cell lines. Neuroblastoma cell line N18 responds to dopamine with a hyperpolarization (Peacock & Nelson, 1973), and the hybrid cell NG108-15 responds to dopamine with a depolarization (Myers & Livengood, 1975; Traber *et al.* 1975; Myers *et al.* 1977; Christian *et al.* 1978). In the present experiment, a hyperpolarizing response was recorded following the transient depolarizing response in a minority of N1E-115 cells.

The neuroblastoma cell line N1E-115 responds to noradrenaline, but to a lesser extent than dopamine. The ionophoretically induced dopamine current diminishes during bath application of dopamine. Desensitization is less pronounced to bath-applied noradrenaline. The cross desensitization between dopamine and noradrenaline indicates that dopamine and noradrenaline act on common receptors. These phenomena were also observed with the hybrid cell NG108-15 (Myers & Livengood, 1975; Myers *et al.* 1977).

Haloperidol is well known as a dopaminergic antagonist, and [^{3}H]haloperidol has been used as a ligand at nanomolar concentrations (Seeman, Chau-Wong, Tedesco & Wong, 1975; Creese, Burt & Snyder, 1975). In the present study, haloperidol was found to block the dopamine-induced current at low concentrations with an apparent dissociation constant of 40 nM. Phentolamine, an α-adrenergic antagonist, blocks the dopamine-induced current, but is less potent than haloperidol. Propranolol, a β-adrenergic antagonist, has no effect. Therefore, it is likely that the receptor on the neuroblastoma cell is specific for dopamine, as has been suggested for the hybrid cell NG108-15 (Myers *et al.* 1977).

The reversal potential for the dopamine-induced current in the cell line N1E-115 is estimated to be about +14 mV. This is in contrast with the data on the hybrid cell NG108-15 by Myers *et al.* (1975) who determined the reversal potential for dopamine-induced depolarization at −15 mV by extrapolation of the current–voltage relationship. The difference in the reversal potential could be ascribed to the difference in cell lines, or could be due to the difference in the methods used. Extrapolation of the current–voltage relationship could lead to an erroneous value for the reversal potential, if rectification occurs as in the present experiments (e.g. Figs. 6, 7 and 8).

The results of ion-substitution experiments clearly indicate that the ionic channel opened by dopamine in the N1E-115 cell line is permeable to both sodium and potassium, but not to chloride and calcium. The high permeability to sodium and

potassium is reminiscent of the ionic channels of some other preparations such as those associated with the acetylcholine receptors in the frog end-plate (Takeuchi & Takeuchi, 1960), and in the bullfrog sympathetic ganglion cell (Kuba & Nishi, 1971, 1979; MacDermott, Connor, Dionne & Parsons, 1980). Recently, the reversal potential for the acetylcholine-induced fast inward currents in neuroblastoma cells has been determined to be -1 mV (Kato, Quandt & Narahashi, 1981) suggesting that the channels are also permeable to both sodium and potassium.

The ratio of sodium conductance change (Δg_{Na}) to potassium conductance change (Δg_K) during the dopamine action can be calculated from the following equation used by Takeuchi & Takeuchi (1960) for the frog end-plate:

$$I = \Delta g_{Na}(E - E_{Na}) + \Delta g_K(E - E_K) \quad (1)$$

where I refers to the dopamine-induced current, E the membrane potential, and E_{Na} and E_K the equilibrium potentials for sodium and potassium, respectively. The values for E_{Na} and E_K were estimated to be $+45$ mV and -70 mV, respectively, in voltage-clamp experiments with neuroblastoma cells (Quandt & Narahashi, 1979; Moolenaar & Spector, 1978; Kato *et al.* 1981). Thus the ratio $\Delta g_{Na}/\Delta g_K$ is calculated to be 2·7. This value is considerably larger than those for the frog end-plate, i.e. 1·79 by Deguchi & Narahashi (1971) and 1·29 by Takeuchi & Takeuchi (1960).

The results of experiments with altered calcium concentration indicate two significant features. First, unlike the frog end-plate (Takeuchi, 1963) and the bullfrog sympathetic ganglion cell (Koketsu, 1969), no significant calcium conductance increase occurs during the action of dopamine. Secondly, calcium has a modulating action on the dopamine receptor-ionic channel complex. An increase in the calcium concentration decreases the dopamine-induced current with an apparent dissociation constant of 1·3 mM. A similar but less pronounced effect of calcium was observed with the acetylcholine-induced conductance of end-plate (Takeuchi, 1963) and of snail neurones (Kanno, Dunning & Machne, 1976). Three possible mechanisms for the calcium block are conceivable, i.e. block of dopamine receptor, block of ionic channel, and a combination of these two. Although calcium is not permeant to the ionic channel associated with the dopamine receptor as discussed above, it has a dimension comparable to that of sodium. Thus it is likely that calcium ions enter the ionic channel from its external mouth but bind to a site within the channel thereby preventing permeant cations such as sodium and potassium from going through.

The ability of calcium in modulating the dopamine response at such low concentrations raises the possibility that it plays a significant role in dopamine-mediated neural functions. An increase in calcium concentration from the normal value of 1·8 to 2·0 mM will cause a 12 % decrease in dopamine-induced current (Fig. 9), an amount of change that will significantly modify synaptic transmission at dopaminergic junctions.

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