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# Effects of dopamine on snail neurones

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**Abstract**

The pharmacological features of dopamine receptors in identifiable giant neurone types of a snail (*Achatina fulica* Férussac) were studied. Under voltage clamp, two neurone types, LVMN (left ventral multiple spike neurone) and d-RPeAN (dorsal-right pedal anterior neurone), produced an inward current ($I_{in}$) in response to dopamine, (−)-noradrenaline and epinine, whereas v-LCDN (ventral-left cerebral distinct neurone) produced an outward current ($I_{out}$) in response to dopamine and epinine. Mammalian dopamine receptor agonists, fenoldopam (dopamine $D_1$-like receptor agonist), (±)-SKF 38393 (1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol) ($D_1$-like), apomorphine ($D_2$-like), (−)-quinpirole ($D_3$ and $D_4$) and methylergometrine showed slight or no effect. (±)-SKF 83566 ((±)-7-bromo-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) (dopamine $D_1$-like receptor antagonist) and (+)-UH 232 (*cis*-(+)-5-methoxy-1-methyl-2-(di-*n*-propylamino)tetralin) ($D_3$ and $D_2$) non-competitively inhibited the $I_{in}$ of LVMN and d-RPeAN, but (±)-sulpiride ($D_2$-like) was without effect. In contrast, (±)-sulpiride competitively inhibited $I_{out}$ of v-LCDN, (+)-UH 232 non-competitively inhibited $I_{out}$ of v-LCDN but (±)-SKF 83566 was without effect. H-7 (1-(5-isoquinolinesulfonyl)-2-methylpiperazine) (non-selective protein kinase inhibitor) inhibited $I_{in}$ of LVMN and d-RPeAN, but did not affect $I_{out}$ of v-LCDN. Dopamine-induced $I_{in}$ was $Na^+$-dependent; $I_{out}$ was $K^+$-dependent. Ouabain did not affect these currents. We propose that the pharmacological features of *Achatina* neuronal dopamine receptors are not fully comparable to those of mammals, although intracellular signal transduction systems linked with dopamine receptors may similarly exist in different animal species.

*Keywords:* Catecholamine; Dopamine; Dopamine receptor, agonist, antagonist; Protein kinase inhibitor; Ouabain; Ionic mechanism; Voltage clamp; Neuron; (Snail)

## 1. Introduction

Some identifiable giant neurones of an African giant snail (*Achatina fulica* Férussac) are sensitive to dopamine and its analogues. The effects of catecholamines and monophenolamines, applied by bath, on these neurones have been examined, to classify their dopamine receptors into subtypes (Ku and Takeuchi, 1985, 1986). Ergot alkaloids, including ergometrine and methylergometrine, applied by bath, showed either excitatory or inhibitory effects on these neurones perhaps as dopamine agonists (Miyamoto et al., 1979, 1980; Ku and Takeuchi, 1983).

The mammalian dopamine receptors have been classified by gene cloning into five subtypes, dopamine $D_1$, $D_2$, $D_3$, $D_4$ and $D_5$. The pharmacological features of dopamine $D_1$ and $D_5$ receptors are similar, and those of dopamine $D_2$, $D_3$ and $D_4$ receptors are also similar. The stimulation of the dopamine $D_1$ and $D_5$ receptors activates adenylate cyclase, stimulation of the dopamine $D_2$ receptor inhibits this enzyme, and the effect of stimulation of the dopamine $D_3$ and $D_4$ receptors has not yet been characterized. Therefore, the dopamine $D_1$ and $D_5$ receptors are termed dopamine $D_1$-like receptors, whereas the dopamine $D_2$, $D_3$ and $D_4$ receptors are termed dopamine $D_2$-like receptors (Sokoloff et al., 1990; Van Tol et al., 1991; Sunahara et al., 1991; Gingrich and Caron, 1993).

The present study aimed to elucidate the characteristics of the dopamine receptors in the *Achatina* giant neurones by using dopamine analogues and mammalian dopamine receptor agonists and antagonists under voltage clamp conditions. The results obtained were compared with those for mammalian dopamine

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receptors. To prevent transsynaptic influences as much as possible, dopamine and the following dopamine analogues and mammalian dopamine receptor agonists were applied locally onto the neurone tested by brief pneumatic pressure ejection: (−)-noradrenaline, (−)-adrenaline, epinine, (±)-octopamine, (±)-synephrine, fenoldopam (mammalian dopamine $D_1$-like receptor agonist) (Hahn et al., 1982; Ohlstein et al., 1984; Alkadhi et al., 1986; Sunahara et al., 1991), (±)-SKF 38393 (dopamine $D_1$-like receptor agonist) (Setler et al., 1978; Sibley et al., 1982; Lang and Woodman, 1982; Hu and Wang, 1988; Sunahara et al., 1991), apomorphine (dopamine $D_2$-like receptor agonist) (Creese et al., 1983; Sokoloff et al., 1990; Van Tol et al., 1991), (−)-quinpirole (dopamine $D_3$ and $D_4$ receptor agonist) (Hahn and MacDonald, 1984; Sokoloff et al., 1990; Van Tol et al., 1991; Momiyama et al., 1993) and methylergometrine (Andrews and Woodruff, 1982). The effects of the following mammalian dopamine receptor antagonists on the dopamine-induced responses were also examined: (±)-SKF 83566 (dopamine $D_1$-like receptor antagonist) (O'Boyle and Waddington, 1985; Molloy and Waddington, 1985; Sunahara et al., 1991), (±)-sulpiride (dopamine $D_2$-like receptor antagonist) (Trabucchi et al., 1975; Sokoloff et al., 1990; Van Tol et al., 1991) and (+)-UH 232 (dopamine $D_3$ and $D_2$ receptor antagonist) (Svensson et al., 1986; Sokoloff et al., 1990; Waters et al., 1993). It has been demonstrated that the adenylate cyclase coupled to GTP-binding protein is activated by dopamine in snail neurones (Osborne, 1977; Deterre et al., 1986). The effects of a non-selective protein kinase inhibitor (inhibitor of protein kinases A, G and C), H-7 (Hidaka et al., 1984), on the responses to dopamine were examined. The ionic dependence of dopamine effects was analyzed. To determine the ATPase involvement with the responses to dopamine, the effects of ouabain on these responses were also examined.

## 2. Materials and methods

### 2.1. Preparations

African giant snails (*Achatina fulica* Férussac) were brought by air from Cebu and Manila, Philippines. The suboesophageal or cerebral ganglia were dissected out and incubated with 0.67% trypsin (Type III, Sigma Chemical, USA) for 5–10 min at room temperature, 21 ± 1°C, to soften the covering connective tissue. The ganglia were fixed on the Sylgard layer in the experimental chamber (about 0.2 ml in volume) by a suction pipette. The connective tissue was carefully removed with fine tweezers under a binocular microscope, to denude the neurones to be tested.

The screening trials for the dopamine-sensitive neurone types were performed on the 25 identifiable giant neurone types. After that, three types of neurones, LVMN (left visceral multiple spike neurone), d-RPeAN (dorsal-right pedal anterior neurone) and v-LCDN (ventral-left cerebral distinct neurone), were selected for further experiments. Their localization in the ganglia, axonal pathways and sensitivities to the small molecule putative neurotransmitters and their analogues, applied by bath, have been reported previously (Takeuchi et al., 1985a, b, c, 1987, 1988; Goto et al., 1986; Yongsiri et al., 1986).

### 2.2. Electrophysiological arrangements

The mapping study of the dopamine-sensitive neurone types was performed under current clamp using an intracellular glass microelectrode of 2–5 MΩ, filled with 2 M potassium acetate. The rest of the experiments were carried out under voltage clamp conditions using two microelectrodes of the same type (Okamoto et al., 1976). The holding voltage ($V_h$) of the neuromembrane was set mainly at −50 mV, near to the resting potentials of these neurones. The membrane potentials and currents were recorded with a pen-writing galvanometer and stored on video tapes via an A-D signal converter. The membrane conductance was measured by superimposing the repetitive hyperpolarizing square pulses (5 mV, 1 s and 0.5 Hz) on the $V_h$.

### 2.3. Compounds used

Dopamine hydrochloride was obtained commercially from Wako Chemicals (Japan), (−)-noradrenaline hydrochloride, (−)-adrenaline bitartrate, epinine hydrochloride, (±)-octopamine hydrochloride, (±)-synephrine, apomorphine hydrochloride and ouabain octahydrate from Sigma Chemical, (±)-SKF 38393 hydrochloride (1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol hydrochloride), (−)-quinpirole hydrochloride (LY 171555, *trans*-(−)-4*aR*-4,4*a*,5,6,7,8,8*a*,9-octahydro-5-propyl-1*H*-pyrazolo[3,4-*g*]quinoline hydrochloride) and (±)-SKF 83566 hydrochloride ((±)-SCH 24543, (±)-7-bromo-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride) from Research Biochemical (USA), and H-7 (1-(5-isoquinolinesulfonyl)-2-methylpiperazine) from Seikagaku Corporation (Japan).

Fenoldopam mesylate (SKF 82526, 6-chlor-2,3,4,6-tetrahydro-1-(*p*-hydroxyphenyl)-1*H*-3-benzazepine-7,8-diolmesylate), prepared by SmithKline Beecham Pharmaceuticals (UK), was donated by Dr. R.R. Luther of Neurex Corporation (USA), methylergometrine maleate by Sandoz (Switzerland), (±)-sulpiride by Fujisawa Pharmaceutical (Japan), and (+)-UH 232 (*cis*-(+)-5-methoxy-1-methyl-2-(di-*n*-propylamino)tetralin) by Dr. M.F. Piercey of Upjohn Laboratories (USA).

### 2.4. Application of compounds

The *Achatina* physiological solution was formulated according to the amounts of principal inorganic ions in the hemolymph as follows (Takeuchi et al., 1973) (mM): NaCl (65.6), KCl (3.3), $CaCl_2$ (10.7), $MgCl_2$ (13.0), Tris-HCl (9.0) and Tris base (1.0) (pH = 7.5). Dopamine, its analogues and mammalian dopamine receptor agonists were dissolved in this solution at $10^{-2}$ M together with 0.5% Fast Green, loaded into a glass micropipette, and applied locally onto the neurone tested by brief pneumatic pressure ejection (mainly $2 \times 10^5$ Pa, 400 ms and 5–10 min interval) under a constant flow (2–3 ml/min) of the physiological solution. Fenoldopam, (±)-SKF 38393, apomorphine and (−)-quinpirole at $10^{-2}$ M were dissolved in the physiological solution containing 1% dimethyl sulfoxide (DMSO). It was confirmed that the brief pressure ejection of 1% DMSO alone had no effect on these neurones and that the similar ejection of dopamine at $10^{-2}$ M, dissolved in 1% DMSO, showed normal effects. Mammalian dopamine receptor agonists were also applied in the bath at $10^{-4}$ M. Mammalian dopamine receptor antagonists at $3 \times 10^{-5}$–$10^{-6}$ M, H-7 and ouabain at $10^{-4}$ M were perfused in the experimental chamber by the same constant flow as described above.

In the experiments involving extracellular ion substitution, the $Na^+$-free solution was prepared by the replacement of $Na^+$ with $Tris^+$. The modification of $K^+$ concentrations was made simply by adjusting the quantity of $K^+$. A $Cl^-$-free solution was made by the proportional replacement of $Cl^-$ with $acetate^-$.

### 2.5. Statistics

The results are presented as the mean values ± standard error of the means (S.E.M.) for $n$ trials. The multiple data obtained from the different neurones were compared by the one-way analysis of variance (one-way ANOVA) and Bonferroni's $t$-test. The multiple data obtained by the repeated measurements from one neurone were compared by ANOVA for repeated measurements and Bonferroni's $t$-test (Glantz, 1987). The two data obtained from one neurone were compared by the two-tailed Student's $t$-test for paired data. Results were considered to be significantly different when $P < 0.05$.

Dose (pressure duration)-response curves were analyzed by the probit method (Litchfield and Wilcoxon, 1949) using a computer program. The $ED_{50}$ value (95% confidence limit), the ideal sigmoidal curve ($r$ value) and Hill coefficient ($r$ value) were calculated in this way. The linear and quadratic regressions were calculated by computer programs.

## 3. Results

### 3.1. Achatina neurone types sensitive to dopamine

To prevent transsynaptic influences, dopamine was applied locally to the neurone by brief pneumatic pressure ejection ($2 \times 10^5$ Pa, 400 ms and $10^{-2}$ M) under current clamp. Among 25 giant neurone types tested, three, LVMN, RVMN and d-RPeAN, were markedly depolarized by dopamine, and two, v-LCDN and v-RCDN, were markedly hyperpolarized and four, d-LCDN, d-RCDN, RPeNLN and LPeNLN, were slightly hyperpolarized; one, PON, responded variably, and the remaining 15, TAN, TAN-2, TAN-3, RAPN, d-RPLN, INN, VIN, d-VLN, v-RPLN, v-VLN, v-VNAN, v-VAN, v-LPSN, d-LPeCN and d-LPeLN, were unaffected. The full names of these neurone types have been described in previous reports (Takeuchi et al., 1985a, b, c, 1987, 1988).

Table 1
The currents (mean ± S.E.M. in nA) of the three *Achatina* giant neurone types

| No. | Compound | LVMN ($I_{in}$) | d-RPeAN ($I_{in}$) | v-LCDN ($I_{out}$) |
|---|---|---|---|---|
| 1. | Dopamine | 11.2 ± 0.7 (44) | 5.1 ± 0.4 (47) | 1.2 ± 0.1 (61) |
| 2. | (−)-Noradrenaline | 8.3 ± 1.0 (5) | 4.4 ± 0.8 (7) | 0.1 ± 0.1 (6) [b] |
| 3. | (−)-Adrenaline | 3.5 ± 0.7 (5) [a] | 1.5 ± 0.5 (6) [a] | 0.2 ± 0.1 (9) [b] |
| 4. | Epinine | 7.8 ± 2.1 (7) | 3.5 ± 0.6 (7) | 1.7 ± 0.3 (6) |
| 5. | (±)-Octopamine | 0.0 (4) [b] | 0.0 (5) [b] | 0.0 (7) [b] |
| 6. | (±)-Synephrine | 0.0 (4) [b] | 0.0 (4) [b] | 0.0 (7) [b] |
| 7. | Fenoldopam (dopamine $D_1$-like receptor agonist) | 0.3 ± 0.1 (4) [b] | 0.0 (5) [b] | 0.2 ± 0.0 (4) [b] |
| 8. | (±)-SKF 38393 (dopamine $D_1$-like receptor agonist) | 0.7 ± 0.2 (4) [b] | 0.4 ± 0.1 (5) [b] | 0.0 (4) [b] |
| 9. | Apomorphine (dopamine $D_2$-like receptor agonist) | 0.5 ± 0.0 (4) [b] | 0.1 ± 0.0 (5) [b] | 0.0 (4) [b] |
| 10. | (−)-Quinpirole (dopamine $D_3$ and $D_4$ receptor agonist) | 0.3 ± 0.0 (4) [b] | 0.3 ± 0.1 (4) [b] | 0.2 ± 0.0 (5) [b] |
| 11. | Methylergometrine | 0.0 (5) [b] | 0.0 (4) [b] | 0.0 (4) [b] |

LVMN, d-RPeAN and v-LCDN, produced by dopamine, dopamine analogues and mammalian dopamine receptor agonists, applied by the brief pneumatic pressure ejection ($2 \times 10^5$ Pa, 400 ms, $10^{-2}$ M and 10-min intervals). The numbers of trials are indicated in parentheses. LVMN, left visceral multiple spike neurone; d-RPeAN, dorsal-right pedal anterior neurone; v-LCDN, ventral-left cerebral distinct neurone; $I_{in}$, inward current; $I_{out}$, outward current. The values obtained from each neurone type were compared with the respective dopamine-induced current by one-way ANOVA and Bonferroni's $t$-test ([a]$P < 0.01$ and [b]$P < 0.001$).

It has been demonstrated that the sensitivities to the small molecule putative neurotransmitters of LVMN are similar to those of RVMN, and those of v-LCDN to those of v-RCDN (Takeuchi et al., 1985a, b). Therefore, LVMN and d-RPeAN, which were excited by dopamine, and v-LCDN, which was inhibited by dopamine, were selected for the following experiments.

### 3.2. Effects of dopamine under voltage clamp

The brief pneumatic pressure ejection ($2 \times 10^5$ Pa, 400 ms, $10^{-2}$ M and 5–10 min interval) of dopamine produced inward currents ($I_{in}$) in LVMN and d-RPeAN and an outward current ($I_{out}$) in v-LCDN under voltage clamp. These current values are described in Table 1.

The membrane conductance ($g$) values (mean ± S.E.M.) of LVMN ($n = 7$) and d-RPeAN ($n = 8$) in the physiological solution were 0.293 ± 0.066 $\mu$s and 0.334 ± 0.041 $\mu$s, respectively. The duration of $I_{in}$ in these neurone types caused by dopamine was too short to measure the $g$ change during the $I_{in}$. The $g$ values for v-LCDN ($n = 10$) in the physiological solution (control) and during the $I_{out}$ caused by dopamine were 0.142 ± 0.013 $\mu$s and 0.191 ± 0.014 $\mu$s, ($P < 0.001$, compared with the control $g$ value by Student's $t$-test for paired data), respectively, indicating that the $g$ values were significantly augmented during the $I_{out}$.

The current values of the three neurone types produced by dopamine, ejected repeatedly by a brief pressure pulse at 5- to 7-min intervals were stable for more than 65 min ($n = 4$–6) (Figs. 1A, 2A, 3A).

### 3.3. Effects of dopamine analogues and mammalian dopamine receptor agonists

The results obtained are summarized in Table 1. Of the dopamine analogues tested, (−)-noradrenaline and epinine, ejected by the brief pressure pulse, produced $I_{in}$ in LVMN and d-RPeAN; (−)-adrenaline was also effective, but significantly less potent than dopamine. On the other hand, epinine, applied in the same man-

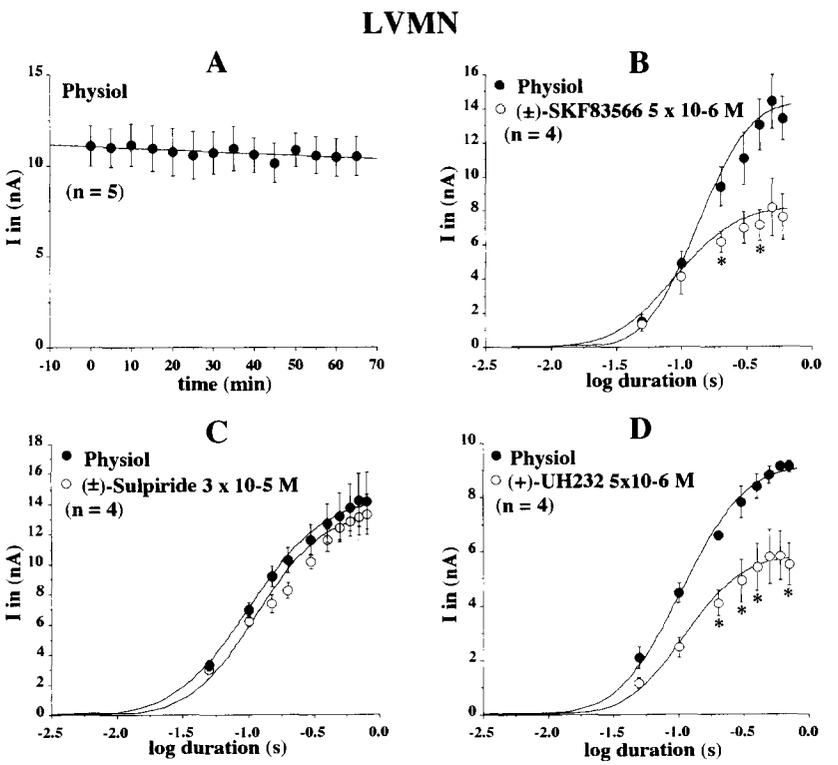


Fig. 1. Effects of mammalian dopamine receptor antagonists on the inward current ($I_{in}$) of LVMN caused by the brief pressure ejection of dopamine. A: Stability of $I_{in}$ caused by repeated dopamine ejection ($2 \times 10^5$ Pa, 400 ms, $10^{-2}$ M and 5-min intervals). Abscissa, time course (min). Ordinate, $I_{in}$ (nA) (small bar: S.E.M.) ($n = 5$). The line was drawn by linear regression. B, C and D: Dose (pressure duration)-response curves of dopamine ($2 \times 10^5$ Pa, varied durations, $10^{-2}$ M and 5-min intervals) in the physiological solution (the control dopamine curve) and under the perfusion of each of the following antagonists (the drug dopamine curve): (±)-SKF 83566 at $5 \times 10^{-6}$ M ($n = 4$) (B), (±)-sulpiride at $3 \times 10^{-5}$ M ($n = 4$) (C) and (+)-UH 232 at $5 \times 10^{-6}$ M ($n = 4$) (D). Abscissa, pressure duration in logarithmic scale (s). Ordinate, $I_{in}$ (nA) (small bar: S.E.M.). A control dopamine curve and a drug dopamine curve were obtained from one neurone. The $I_{in}$ values of the drug dopamine curve were compared with the corresponding values of the control dopamine curve by Student's $t$-test for paired data ($^*P < 0.05$). These curves were drawn by fitting data to ideal sigmoidal curves calculated by a computer program ($r$ values = 0.982626 (B, control dopamine curve), 0.992086 (B, drug dopamine curve), 0.978316 (C, control), 0.995481 (C, drug), 0.991065 (D, control) and 0.9851 (D, drug)). $V_h = -50$ mV.

ner, produced $I_{out}$ in v-LCDN; (−)-noradrenaline and (−)-adrenaline were effective, but much less potent than dopamine. (±)-Octopamine and (±)-synephrine showed no effect on any of the neurone types tested.

Among the mammalian dopamine receptor agonists tested, fenoldopam, (±)-SKF 38393, apomorphine and (−)-quinpirole produced a slight $I_{in}$ in LVMN (less than 10% of the dopamine effect); (±)-SKF 38393, apomorphine and (−)-quinpirole caused a slight $I_{in}$ in d-RPeAN (also less than 10%); and fenoldopam and (−)-quinpirole produced a small $I_{out}$ in v-LCDN (less than 20% of dopamine effects). The rest had no effect.

Bath application of these dopamine agonists at $10^{-4}$ M on these neurone types showed only a slight or no effect ($n = 4$ for all), as they did when ejected by the brief pressure pulse.

### 3.4. Effects of mammalian dopamine receptor antagonists

The effects of the three mammalian dopamine receptor antagonists, (±)-SKF 83566, (±)-sulpiride and (+)-UH 232, on the dose-response curve of dopamine (the dopamine curve) were examined. For this purpose, the two dose-response curves of dopamine, applied by brief pressure ejection, were measured by varying the pressure duration from 50 ms to 500–800 ms, in the physiological solution (the control dopamine curve) and under the perfusion of an antagonist (the drug dopamine curve) from one neurone. The results obtained are summarized in Table 2.

The $I_{in}$ values of LVMN caused by dopamine, ejected repeatedly by a brief pressure pulse ($2 \times 10^5$ Pa, 400 ms and $10^{-2}$ M) at 5-min intervals, were stable at least for 65 min; the relations between the time course (abscissa, min) and the $I_{in}$ (ordinate, nA) were $Y = 11.0540 - 0.009912\ X$ ($n = 5$) (Fig. 1A).

(±)-SKF 83566 at $5 \times 10^{-6}$ M inhibited non-competitively the dose (pressure duration)-response curve of dopamine, applied by pressure ejection, on LVMN. The control dopamine curve and the drug dopamine curve, measured under physiological solution and under (±)-SKF 83566 respectively, were analyzed by probit method as follows ($n = 4$): $ED_{50}$ (95% confidence

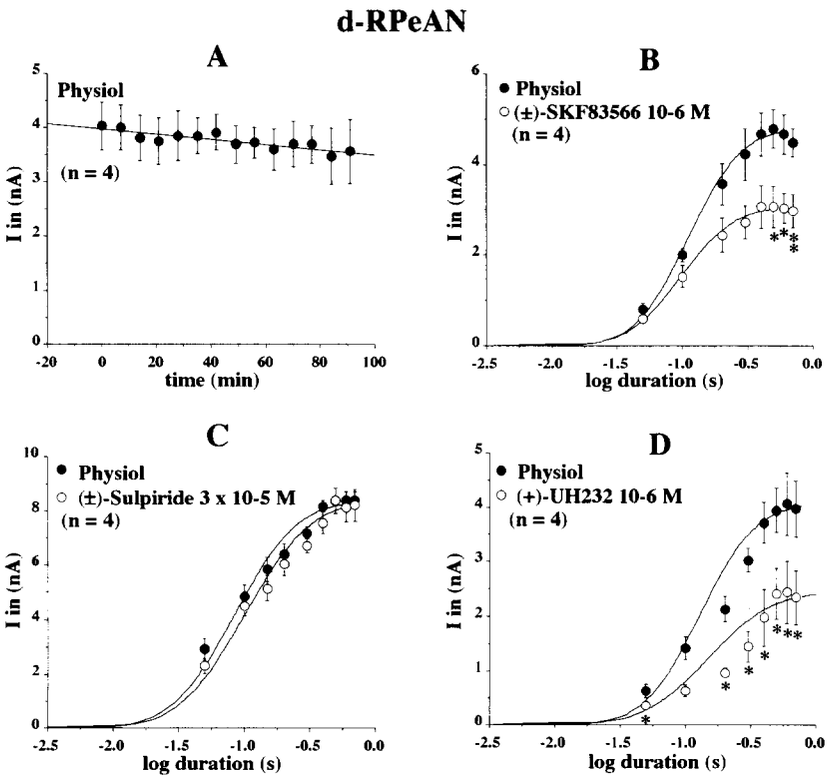


Fig. 2. Effects of mammalian dopamine receptor antagonists on the $I_{in}$ of d-RPeAN caused by dopamine ($n = 4$ for all cases). A: Stability of $I_{in}$ caused by repeated dopamine ejection ($2 \times 10^5$ Pa, 400 ms, $10^{-2}$ M and 7 min interval). Abscissa, time course (min). Ordinate, $I_{in}$ (nA) (small bar: S.E.M.). The line was drawn by linear regression. B, C and D: Dose (pressure duration)-response curves of dopamine ($2 \times 10^5$ Pa, varied durations, $10^{-2}$ M and 7-min intervals) in the physiological solution (the control dopamine curve) and under the perfusion of each of the following antagonists (the drug dopamine curve): (±)-SKF 83566 at $10^{-6}$ M (B), (±)-sulpiride at $3 \times 10^{-5}$ M (C) and (+)-UH 232 at $10^{-6}$ M (D). Abscissa, pressure duration in logarithmic scale (s). Ordinate, $I_{in}$ (nA) (small bar: S.E.M.). A control dopamine curve and a drug dopamine curve were obtained from one neurone. The $I_{in}$ values of the drug dopamine curve were compared with the corresponding value of the control dopamine curve by Student's $t$-test for paired data (** $P < 0.01$). These curves were drawn by fitting data to ideal sigmoidal curves calculated by a computer program ($r$ values = 0.989541 (B, control dopamine curve), 0.983316 (B, drug dopamine curve), 0.963392 (C, control), 0.946104 (C, drug) and 0.957643 (D, control) and 0.894791 (D, drug)). $V_h = -50$ mV.

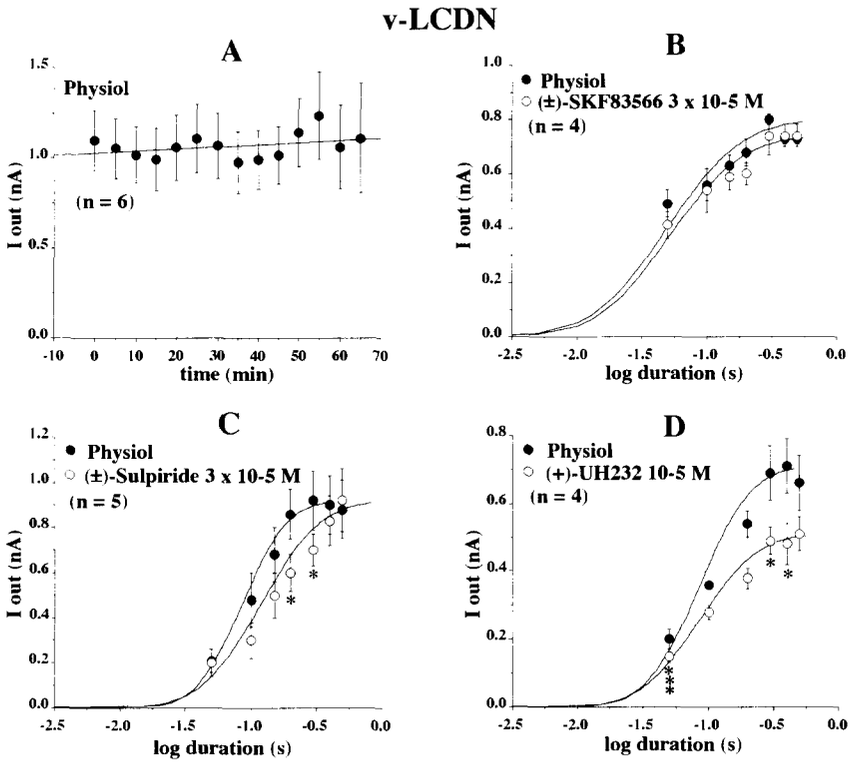


Fig. 3. Effects of mammalian dopamine receptor antagonists on the outward current ($I_{out}$) of v-LCDN caused by dopamine. A: Stability of $I_{out}$ caused by repeated dopamine ejection ($2 \times 10^5$ Pa, 400 ms, $10^{-2}$ M and 5-min intervals). Abscissa, time course (min). Ordinate, $I_{out}$ (nA) (small bar: S.E.M.) ($n = 6$). The line was drawn by linear regression. B, C and D: Dose (pressure duration)-response curves of dopamine ($2 \times 10^5$ Pa, varied durations, $10^{-2}$ M and 5-min intervals) in the physiological solution (the control dopamine curve) and under the perfusion of each of the following antagonists (the drug dopamine curve): (±)-SKF 83566 at $3 \times 10^{-5}$ M ($n = 4$) (B), (±)-sulpiride at $3 \times 10^{-5}$ M ($n = 5$) (C) and (+)-UH 232 at $10^{-5}$ M ($n = 4$) (D). Abscissa, pressure duration in logarithmic scale (s). Ordinate, $I_{out}$ (nA) (small bar: S.E.M.). A control dopamine curve and a drug dopamine curve were obtained from one neurone. The $I_{out}$ values of the drug dopamine curve were compared with the corresponding values of the control dopamine curve by Student's $t$-test for paired data (* * * $P < 0.001$). These curves were drawn by fitting data to ideal sigmoidal curves calculated by a computer program ($r$ values = 0.879798 (B, control dopamine curve), 0.890953 (B, drug dopamine curve), 0.976822 (C, control), 0.962314 (C, drug), 0.973406 (D, control) and 0.95348 (D, drug)). $V_h = -50$ mV.

limit), Hill coefficient ($r$ value) and $E_{max}$ were: 134.4 ms (107.3–168.3 ms), 2.47434 (0.969158) and 14.38 ± 1.57 nA, respectively, for the control dopamine curve; and 89.6 ms (77.7–103.4 ms), 2.10111 (0.984177) and 8.19 ± 1.70 nA, respectively, for the drug dopamine curve. (±)-Sulpiride at $3 \times 10^{-5}$ M did not affect the dopamine curve ($n = 4$): 115 ms (98–135 ms), 1.98996 (0.959876) and 13.31 ± 1.34 nA, respectively, for the control dopamine curve; and 101 ms (93.6–109 ms), 1.70145 (0.989113) and 14.25 ± 1.76 nA, respectively, for the drug dopamine curve. (+)-UH 232 at $5 \times 10^{-6}$ M inhibited non-competitively the dopamine curve ($n$

Table 2
Characteristics of the inhibitory effects of mammalian dopamine receptor antagonists on the dose (pressure duration)-response curves of dopamine ($2 \times 10^5$ Pa, varied duration, $10^{-2}$ M and 5- to 7-min intervals), and effects of a protein kinase (PK) inhibitor on the currents produced by dopamine ($2 \times 10^5$ Pa, 400 ms, $10^{-2}$ M and 10 min interval)

| No. | Compound | Concentrations | LVMN ($I_{in}$) | d-RPeAN ($I_{in}$) | v-LCDN ($I_{out}$) |
|---|---|---|---|---|---|
| 1. | (±)-SKF 83566 (dopamine $D_1$-like receptor antagonist) | $3 \times 10^{-5}$–$10^{-6}$ M | Non-competitive (4) | Non-competitive (4) | Ineffective (4) |
| 2. | (±)-Sulpiride (dopamine $D_2$-like receptor antagonist) | $3 \times 10^{-5}$ M | Ineffective (4) | Ineffective (4) | Competitive (5) |
| 3. | (+)-UH 232 (dopamine $D_3$ and $D_2$ receptor antagonist) | $10^{-5}$–$10^{-6}$ M | Non-competitive (4) | Non-competitive (4) | Non-competitive (4) |
| 4. | H-7 (inhibitor of PKA, PKG and PKC) | $10^{-4}$ M | Inhibitory (4) | Inhibitory (6) | Ineffective (4) |

The numbers of trials are indicated in parentheses. $I_{in}$, inward current; $I_{out}$, outward current. Effects of H-7 on the dose-response curves of dopamine were not examined.

= 4): 105.1 ms (92.2–119.7 ms), 2.0326 (0.978911) and 9.13 ± 0.14 nA, respectively, for the control dopamine curve; and 110.8 ms (91.0–134.9 ms), 2.20936 (0.96996) and 5.81 ± 0.90 nA, respectively, for the drug dopamine curve (Fig. 1B–D).

The $I_{in}$ values of d-RPeAN caused by dopamine, ejected repeatedly by the brief pressure pulse at 7-min intervals, were stable for at least 91 min; $Y$ ($I_{in}$, nA) = 3.9759 − 0.004812 $X$ (time, min) ($n$ = 4) (Fig. 2A).

(±)-SKF 83566 at $10^{-6}$ M inhibited non-competitively the dopamine curve of d-RPeAN ($n$ = 4): $ED_{50}$ (95% confidence limit), Hill coefficient ($r$ value) and $E_{max}$ were 110.9 ms (94.1–130.8 ms), 2.43154 (0.978778) and 4.78 ± 0.42 nA, respectively, for the control dopamine curve; and 97.4 ms (73.9–127.6 ms), 2.3962 (0.967198) and 3.06 ± 0.46 nA, respectively, for the drug dopamine curve. (±)-Sulpiride at $3 \times 10^{-5}$ M did not affect the dopamine curve ($n$ = 4): 84.3 ms (63.7–107.3 ms), 2.14183 (0.940910) and 8.38 ± 0.32 nA, respectively, for the control dopamine curve; and 100.9 ms (73.2–139.3 ms), 1.97705 (0.914307) and 8.38 ± 0.46 nA, respectively, for the drug dopamine curve. (+)-UH 232 at $10^{-6}$ M inhibited non-competitively the dopamine curve ($n$ = 4): 129.2 ms (92.1–201.8 ms), 2.29355 (0.941347) and 4.06 ± 0.57 nA, respectively, for the control dopamine curve; and 147.8 ms (83.4–540.5 ms), 1.98548 (0.866676) and 2.44 ± 0.57 nA, respectively, for the drug dopamine curve (Fig. 2B–D).

The $I_{out}$ of v-LCDN caused by dopamine, ejected repeatedly by the brief pressure pulse at 5-min intervals, were stable for at least 65 min; $Y$ ($I_{out}$, nA) = 1.0287 + 0.001142 $X$ (time, min) ($n$ = 6) (Fig. 3A).

(±)-SKF 83566 at $3 \times 10^{-5}$ M did not affect the dopamine curve of v-LCDN ($n$ = 4): $ED_{50}$ (95% confidence limit), Hill coefficient ($r$ value) and $E_{max}$ were 49.5 ms (−91.5 ms), 1.82648 (0.852185) and 0.80 ± 0.02 nA, respectively, for the control dopamine curve; and 51.6 ms (−92.0 ms), 1.86755 (0.860281) and 0.74 ± 0.04 nA, respectively, for the drug dopamine curve. (±)-Sulpiride at $3 \times 10^{-5}$ M inhibited competitively the dopamine curve of v-LCDN ($n$ = 5): 85.2 ms (45.3–124.0 ms), 2.69402 (0.966993) and 0.92 ± 0.13 nA, respectively, for the control dopamine curve; and 118.5 ms (92.7–157.6 ms), 2.12037 (0.94093) and 0.92 ± 0.14 nA, respectively, for the drug dopamine curve. (+)-UH 232 at $10^{-5}$ M inhibited non-competitively the dopamine curve ($n$ = 4): 84.7 ms (56.2–118.4 ms), 2.51598 (0.961467) and 0.71 ± 0.08 nA, respectively, for

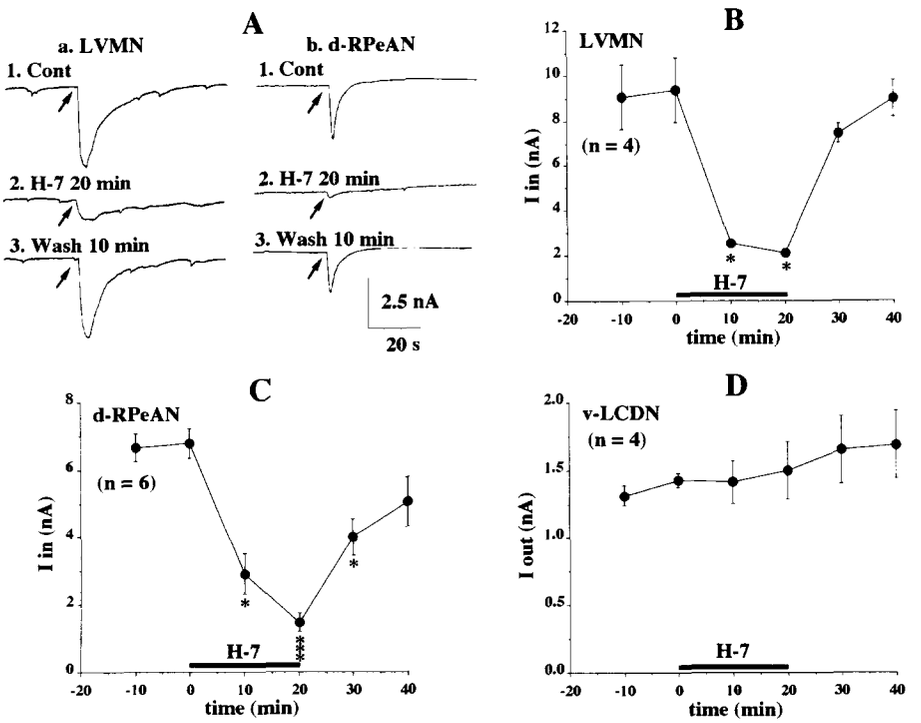


Fig. 4. Effects of a non-selective protein kinase inhibitor, H-7, on currents produced by dopamine, ejected by a brief pressure pulse ($2 \times 10^5$ Pa, 400 ms, $10^{-2}$ M and 10-min intervals). A: Effects of H-7 at $10^{-4}$ M on $I_{in}$ caused by dopamine of LVMN (a) and d-RPeAN (b). Arrows indicate dopamine ejection. B, C and D: Time course of the effects of H-7 at $10^{-4}$ M on the dopamine-induced currents of LVMN ($n$ = 4) (B), d-RPeAN ($n$ = 6) (C) and v-LCDN ($n$ = 4) (D). Abscissa, time course (min); the horizontal bar shows the perfusion time of H-7 at $10^{-4}$ M. Ordinate, currents induced by dopamine (small bar: S.E.M.). The values were compared with the mean of the values obtained before the drug perfusion (the control) by ANOVA for repeated measurements and Bonferroni's $t$-test (*$P < 0.05$; and ***$P < 0.001$). $V_h$ = −50 mV.

the control dopamine curve; and 84.5 ms (0.4–266.4 ms), 2.18113 (0.936626) and 0.51 ± 0.05 nA, respectively, for the drug dopamine curve (Fig. 3B–D).

### 3.5. Effects of protein kinase inhibitor

The effects of a non-selective protein kinase inhibitor, H-7, perfused at $10^{-4}$ M on the currents induced by the brief pressure ejection of dopamine ($2 \times 10^5$ Pa, 400 ms, $10^{-2}$ M and 10 min interval) on the three neurone types were examined. The results obtained are summarized in Table 2.

H-7 inhibited the $I_{in}$ of LVMN caused by dopamine ($n = 4$): 9.22 ± 1.42 nA for the mean of control, 2.56 ± 0.14 nA ($P < 0.05$, compared with the mean of control by ANOVA for repeated measurements and Bonferroni's $t$-test) 10 min after the drug perfusion, 2.13 ± 0.06 nA 20 min after ($P < 0.05$), and 7.44 ± 0.43 nA (not significant, NS) 10 min after washout. This compound also inhibited the $I_{in}$ of d-RPeAN caused by dopamine ($n = 6$): 6.73 ± 0.42 nA for the mean of control, 2.92 ± 0.60 nA ($P < 0.05$) 10 min after the drug, 1.48 ± 0.27 nA ($P < 0.001$) 20 min after, and 5.06 ± 0.73 nA (NS) 20 min after washout. This compound did not affect the $I_{out}$ of v-LCDN ($n = 4$): 1.37 ± 0.06 nA for the mean of control, 1.41 ± 0.16 nA (NS) 10 min after the drug, and 1.50 ± 0.21 nA (NS) 20 min after (Fig. 4).

### 3.6. Ionic dependence

The $I_{in}$ values of LVMN and d-RPeAN caused by dopamine, ejected by the brief pressure pulse, at $V_h$ of −50 mV were markedly decreased in the $[Na^+]_o$-free medium, indicating that the $I_{in}$ was $Na^+$-dependent. The $I_{in}$ of LVMN ($n = 4$) was 8.47 ± 1.45 nA for the control and 1.84 ± 0.85 nA($P < 0.01$, compared with the control by Student's $t$-test for paired data) 10 min after perfusion with $[Na^+]_o$-free medium. The $I_{in}$ of d-RPeAN ($n = 4$) was 7.31 ± 1.32 nA for the control and 1.38 ± 0.40 nA ($P < 0.05$) 10 min after perfusion with the $[Na^+]_o$-free medium (Fig. 5A).

The reversal potentials of the current in v-LCDN produced by dopamine, applied in the same manner

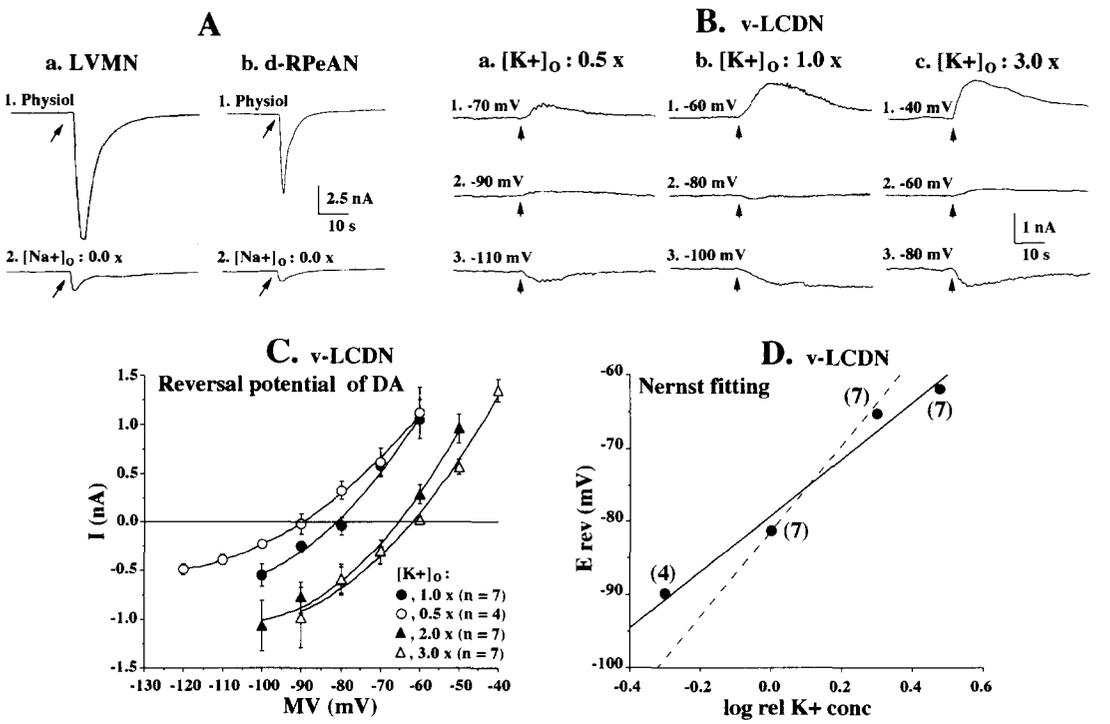


Fig. 5. Ionic dependence of the currents induced by dopamine, ejected by a brief pressure pulse ($2 \times 10^5$ Pa, 400 ms, $10^{-2}$ M and 10-min intervals). A: Influence of $[Na^+]_o$-free medium on the $I_{in}$ of LVMN (a) and d-RPeAN (b) caused by dopamine. $V_h = -50$ mV. B: Current of v-LCDN produced by dopamine at different $V_h$ in varied $[K^+]_o$. a: Half (0.5 ×) of the normal $[K^+]_o$ (1.65 mM). b: Normal (1.0 ×) $[K^+]_o$ (3.3 mM). c: Three times (3.0 ×) of the normal $[K^+]_o$ (9.9 mM). $V_h$ were varied from −40 mV to −110 mV, as shown in the figure. In A and B, arrows indicate dopamine ejection. C: Reversal potential of the current caused by dopamine ($E_{DA}$) in varied $[K^+]_o$. Abscissa, $V_h$ (mV). Ordinate, current induced by dopamine (nA) (small bar: S.E.M.). The relative $[K^+]_o$ values and the number of experiments are shown in the figure. These curves were drawn by the quadratic regression ($r = 0.996$ ($[K^+]_o$: 1.0 ×), 0.999 (0.5 ×), 0.996 (2.0 ×) and 0.996 (3.0 ×)). D: Comparison of the measured $E_{DA}$ values with those calculated by the Nernst equation as $E_{DA} = E_K$. Abscissa, relative $[K^+]_o$ value in logarithmic scale (0.0 = normal $[K^+]_o$ (3.3 mM)). Ordinate, $E_{DA}$ (mV). The numbers of trials are given in parentheses. The smooth line was drawn by the linear regression of the data, shown in C. The dashed line was drawn by the values calculated by the Nernst equation as $E_K$ through the $E_{DA}$ value measured in the normal $[K^+]_o$.

($E_{DA}$), were measured under varied $[K^+]_o$. The $E_{DA}$ values, obtained by the quadratic regression between the $V_h$ and the currents caused by dopamine, in each $[K^+]_o$ were as follows: −89.75 mV in half (0.5 ×) of the normal $[K^+]_o$ (1.65 mM) ($n = 4$), −82.51 mV in the normal (1.0 ×) $[K^+]_o$ (3.3 mM) ($n = 7$), −65.33 mV in twice (2.0 ×) of the normal $[K^+]_o$ (6.6 mM) ($n = 7$) and −61.89 mV in 3 times (3.0 ×) of the normal $[K^+]_o$ (9.9 mM) ($n = 7$) (Fig. 5B, C).

The relations between the relative values of $[K^+]_o$ in logarithmic scale (abscissa) and the measured $E_{DA}$ (ordinate, mV) were obtained by the linear regression: $Y = -79.140 + 38.196\ X$. The same relations calculated with the Nernst equation (at 20°C) as '$E_{DA} = E_K$', passing through $E_{DA}$ value obtained in the normal $[K^+]_o$, were: $Y = -81.229 + 58.106\ X$, which were near to those of the measured $E_{DA}$, indicating that the $I_{out}$ of v-LCDN was mainly $K^+$-dependent (Fig. 5D).

The $E_{DA}$ values of v-LCDN in the $[Cl^-]_o$-free medium ($n = 4$) and the $[Na^+]_o$-free medium ($n = 4$) (normal $[K^+]_o$ for both), measured in the same manner, were −80.0 mV and −81.1 mV, respectively, which were similar to the value measured with the physiological solution, indicating that the $I_{out}$ of this neurone type was due neither to $Cl^-$ nor to $Na^+$. Besides, the $I_{out}$ of v-LCDN caused by dopamine was unaffected in the $[Na^+]_o$-free medium ($n = 3$): 1.12 ± 0.17 nA for the mean of control, and 1.20 ± 0.24 nA (NS) 10 min after the perfusion of the $[Na^+]_o$-free medium, and 1.07 ± 0.19 nA (NS) 20 min after.

### 3.7. Effects of ouabain

The currents of the three neurone types produced by dopamine, ejected in the same manner, were unaffected by ouabain at $10^{-4}$ M. The $I_{in}$ of LVMN ($n = 3$) was 10.67 ± 1.71 nA for the mean of control, 10.50 ± 1.55 nA (NS, compared with the mean of control by ANOVA for repeated measurements and Bonferroni's $t$-test) 10 min after the ouabain perfusion, and 10.92 ± 1.79 nA (NS) 20 min after the perfusion. The $I_{in}$ of d-RPeAN ($n = 3$) was 4.81 ± 0.32 nA for the mean of control, 4.96 ± 0.62 nA (NS) 10 min after the drug, and 4.38 ± 0.79 nA (NS) 20 min after the drug. The $I_{out}$ of v-LCDN ($n = 4$) was 1.09 ± 0.08 nA for the mean of control, 1.09 ± 0.11 nA (NS) 10 min after the drug, and 0.95 ± 0.11 nA (NS) 20 min after the drug.

## 4. Discussion

The local application of dopamine, ejected by a brief pressure pulse, onto the neurone tested produced $I_{in}$ in LVMN and d-RPeAN under voltage clamp conditions. Throughout the present experiments, the features of the $I_{in}$ of the two neurone types were almost identical. In contrast, dopamine, applied in the same manner, caused an $I_{out}$ in v-LCDN. The *Achatina* neuronal dopamine receptors can be classified into excitatory and inhibitory dopamine receptors, as described by Cools and Van Rossum (1980).

The durations of the dopamine-induced $I_{in}$ in LVMN and d-RPeAN were too short to measure the membrane conductance ($g$) of these neurones. However, it could be concluded that the $I_{in}$ was mainly due to the increase in the membrane permeability to $Na^+$, since the $I_{in}$ was markedly reduced in the $[Na^+]_o$-free medium. In contrast, the $g$ value for the v-LCDN membrane was significantly augmented during the dopamine-induced $I_{out}$, and reversal potentials of the $I_{out}$ ($E_{DA}$) in different $[K^+]_o$ were nearly fitted to the values calculated by the Nernst equation as $E_{DA} = E_K$, indicating that the $I_{out}$ was mainly due to the increase in membrane permeability to $K^+$.

H-7, a non-selective protein kinase inhibitor, markedly inhibited the $I_{in}$ of LVMN and d-RPeAN, suggesting that the *Achatina* excitatory dopamine receptors were linked to a protein kinase. This would be protein kinase A or protein kinase G, since the intracellular injection of cyclic AMP and cyclic GMP into *Achatina* giant neurones produces a membrane depolarization, and injection of $IP_3$ causes a hyperpolarization (Liu and Takeuchi, 1993). In contrast, H-7 did not affect the $I_{out}$ of v-LCDN, which makes it less likely that the inhibitory dopamine receptor is involved with the above protein kinases. These findings suggest that the features of the *Achatina* excitatory dopamine receptors appear to be similar to those of the mammalian dopamine $D_1$-like receptors, and the *Achatina* inhibitory dopamine receptors are similar to the mammalian dopamine $D_2$-like receptors. However, further investigations are needed to elucidate in detail the intracellular signal transduction systems involved with the *Achatina* dopamine receptors.

However, both the excitatory and inhibitory *Achatina* dopamine receptors showed only slight or no response to the following mammalian dopamine receptor agonists: fenoldopam (dopamine $D_1$-like receptor agonist), (±)-SKF 38393 (dopamine $D_1$-like receptor agonist), apomorphine (dopamine $D_2$-like receptor agonist), (−)-quinpirole (dopamine $D_3$ and $D_4$ receptor agonist) and methylergometrine. Their potencies on LVMN and d-RPeAN and on v-LCDN were less than 10% and 20%, respectively, of those of dopamine. In contrast, the potencies of these compounds on the mammalian dopamine receptors were comparable (at least 50%) to those of dopamine, according to the following reports: Setler et al. (1978), Hahn et al. (1982), Lang and Woodman (1982), Alkadhi et al. (1986), Hu and Wang (1988) and Momiyama et al. (1993). Thus there is a major difference between mammalian and *Achatina* dopamine receptors. At first we doubted whether the

contact period of these agonists, ejected by the brief pressure pulse, onto the dopamine receptors, was long enough to activate them. However, this notion was discarded, since these compounds, applied by bath, showed effects similar to those of the same compounds when ejected by the brief pressure pulse onto the *Achatina* neurones.

The effects of the mammalian dopamine receptor antagonists on the *Achatina* dopamine receptors were peculiar, when compared with those on the mammalian dopamine receptors. The $I_{in}$ of LVMN and d-RPeAN produced by dopamine was inhibited non-competitively by (±)-SKF 83566 (mammalian dopamine $D_1$-like receptor antagonist) and (+)-UH 232 (dopamine $D_3$ and $D_2$ receptor antagonist), and unaffected by (±)-sulpiride (dopamine $D_2$-like receptor antagonist). In contrast, the $I_{out}$ of v-LCDN was inhibited competitively by (±)-sulpiride, non-competitively by (+)-UH 232, and not affected by (±)-SKF 83566. Unexpectedly, the major part of the inhibition caused by the mammalian dopamine receptor antagonists of the dopamine effects on the *Achatina* neurones was non-competitive. The inhibition in this manner may be produced not only at the receptor sites, but also at other sites, for example, the ionic channels or the intracellular signal transduction systems.

The dopamine-induced responses of *Achatina* neurones, including the $I_{out}$ of v-LCDN, were insensitive to ouabain, indicating that the ATPase was not involved with these responses, unlike the $K^+$-dependent $I_{out}$ of v-LCDN, caused by β-hydroxy-L-glutamate, which was markedly blocked by ouabain (Zhang and Takeuchi, unpublished data).

The effects of GABA and related compounds on the *Achatina* giant neurone types have been investigated by the same methods as adopted in the present study (Kim and Takeuchi, 1990). The *Achatina* inhibitory GABA receptors have been classified into two subtypes, the muscimol I-type GABA receptors linked with $Cl^-$ channels, similar to the mammalian $GABA_A$ receptors, and the baclofen-type GABA receptors linked to $K^+$ channels, similar to the mammalian $GABA_B$ receptors. The pharmacological characteristics, studied by using GABA agonists and antagonists, of the *Achatina* muscimol I-type GABA receptors are not fully comparable to those of the mammalian $GABA_A$ receptors. The features of the *Achatina* baclofen-type GABA receptors are rather similar to those of the mammalian $GABA_B$ receptors.

With respect to dopamine receptors of other invertebrate species, the growth hormone-producing cells (GHCs) of a fresh water snail, *Lymnaea stagnalis*, are hyperpolarized by dopamine and LY 141865 (dopamine $D_2$-like receptor agonist), slightly hyperpolarized by (−)-quinpirole, and are unaffected by (±)-SKF 38393. The dopamine effects are mediated by a decrease in intracellular cyclic AMP and inhibited by (−)-sulpiride. These *Lymnaea* dopamine receptors would be similar to the mammalian dopamine $D_2$-like receptors (Stoof et al., 1985; De Vlieger et al., 1986; Werkman et al., 1987). Another neurone of the same animal species, RPeD1, is hyperpolarized by dopamine and unaffected by (−)-quinpirole and (±)-SKF 38393. The dopamine effects are slightly inhibited by metaclopramide (mammalian dopamine $D_2$-like receptor antagonist) and unaffected by SCH 23390 (dopamine $D_1$-like receptors antagonist). Further, the B-2 neurone of the same animal species is also hyperpolarized by dopamine, by (±)-SKF 38393 only in case of pressure ejection, but is unaffected by apomorphine and (−)-quinpirole. The dopamine effects on the B-2 neurone are unaffected by SCH 23390 and (±)-sulpiride (Audesirk, 1989). Some neurones of a land snail, *Helix aspersa*, are hyperpolarized by dopamine and very slightly hyperpolarized by (±)-SKF 38393, but are unaffected by (−)-quinpirole. The dopamine effects are inhibited competitively by SCH 23390 and (±)-sulpiride (Holden-Dye and Walker, 1989). In a cockroach (*Periplaneta americana*), a prothoracic inhibitory motoneurone produces an $I_{in}$ in response to dopamine, a small $I_{in}$ in response to apomorphine, and is not affected by fenoldopam and (−)-quinpirole. The $I_{in}$ caused by dopamine was inhibited competitively by SCH 23390 and spiroperidol (dopamine $D_2$-like receptors antagonist) and non-competitively by (±)-sulpiride (Davis and Pitman, 1991). Salivary gland acinar cells (SGACs) of another kind of cockroach (*Nauphoeta cinerea*) are hyperpolarized by dopamine and slightly hyperpolarized by fenoldopam, (±)-SKF 38393 and (−)-quinpirole. The dopamine effects are inhibited by SCH 23390 and unaffected by (±)-sulpiride. The dopamine receptors of SGACs would be similar to the mammalian dopamine $D_2$-like receptors (Evans and Green, 1991). Although these results obtained from several invertebrate species are not fully comparable to those for *Achatina* neurones, the reports cited and our present findings demonstrate that the mammalian dopamine receptor agonists had only slight or no effect on the invertebrate dopamine receptors, except for a few cases.

It can be concluded that the pharmacological features, studied by using agonists and antagonists, of *Achatina* neuronal dopamine receptors are not comparable in detail to those of mammalian receptors, although the intracellular signal transduction systems linked to dopamine receptors may similarly exist in the different animal species.

After elucidation of the pharmacological features of *Achatina* GABA and dopamine receptors and comparison with those of respective mammalian receptors, further studies of small molecule putative neurotransmitters, for example, L-glutamic acid and β-hydroxy-L-

glutamic acid, will enrich our knowledge of the comparative aspects of neurotransmission.

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## References

Alkadhi, K.A., M.H. Sabouni, A.F. Ansari and M.F. Lokhandwala, 1986, Activation of $DA_1$ receptors by dopamine or fenoldopam increases cyclic AMP levels in the renal artery but not in the superior cervical ganglion of the rat, J. Pharmacol. Exp. Ther. 238, 547.

Andrews, C.D. and G.N. Woodruff, 1982, Turning behaviour following nigral injections of dopamine agonists and glycine, Eur. J. Pharmacol. 84, 169.

Audesirk, T.E., 1989, Characterization of pre- and postsynaptic dopamine receptors in *Lymnaea*, Comp. Biochem. Physiol. 93C, 115.

Cools, A.R. and J.M. Van Rossum, 1980, Multiple receptors for brain dopamine in behavior regulation: concept of dopamine-E and dopamine-I receptors, Life Sci. 27, 1237.

Creese, I., D.R. Sibley, M.W. Hamblin and S.E. Leff, 1983, The classification of dopamine receptors: relationship to radioligand binding, Annu. Rev. Neurosci. 6, 43.

Davis, J.P.L. and R.M. Pitman, 1991, Characterization of receptors mediating the actions of dopamine on an identified inhibitory motoneurone of the cockroach, J. Exp. Biol. 155, 203.

Deterre, P., D. Paupardin-Tritsch and J. Bockaert, 1986, Serotonin- and dopamine-sensitive adenylate cyclase in molluscan nervous system. Biochemical and electrophysiological analysis of the pharmacological properties and the GTP-dependence, Mol. Brain Res. 1, 101.

De Vlieger, T.A., J.C. Lodder, J.C. Stoof and T.R. Werkman, 1986, Dopamine receptor stimulation induces a potassium dependent hyperpolarizing response in growth hormone producing neuroendocrine cells of the gastropod mollusc *Lymnaea stagnalis*, Comp. Biochem. Physiol. 83C, 429.

Evans, A.M. and K.L. Green, 1991, Effects of selective $D_1$ and $D_2$ dopamine agonists on cockroach salivary gland acinar cells in vitro, Br. J. Pharmacol. 104, 787.

Gingrich, J.A. and M.G. Caron, 1993, Recent advances in the molecular biology of dopamine receptors, Annu. Rev. Neurosci. 16, 299.

Glantz, S.A., 1987, Primer of Biostatics: The Program (McGraw-Hill Book Co., New York) p. 1.

Goto, T., B.S. Ku and H. Takeuchi, 1986, Axonal pathways of giant neurones identified in the right parietal and visceral ganglia in the suboesophageal ganglia of an African giant snail (*Achatina fuluca* Férussac), Comp. Biochem. Physiol. 83A, 93.

Hahn, R.A. and B.R. MacDonald, 1984, Primate cardiovascular responses mediated by dopamine receptors: effects of *N,N*-di-*n*-propyldopamine and LY 171555, J. Pharmacol. Exp. Ther. 229, 132.

Hahn, R.A., J.R. Wardell, H.M. Sarau and P.T. Ridley, 1982, Characterization of the peripheral and central effects of SK&F 82526, a novel dopamine receptor agonist, J. Pharmacol. Exp. Ther. 223, 305.

Hidaka, H., M. Inagaki, S. Kawamoto and Y. Sasaki, 1984, Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C, Biochemistry 23, 5036.

Holden-Dye, L. and R.J. Walker, 1989, Further characterisation of the dopamine-inhibitory receptor in *Helix* and evidence for a noradrenaline-preferring receptor, Comp. Biochem. Physiol. 93C, 413.

Hu, X.T. and R.Y. Wang, 1988, Comparison of effects of D-1 and D-2 dopamine receptor agonists on neurons in the rat caudate putamen: an electrophysiological study, J. Neurosci. 8, 4340.

Kim, K.H. and H. Takeuchi, 1990, Pharmacological characteristics of two different types of inhibitory GABA receptors on *Achatina fulica* neurones, Eur. J. Pharmacol. 182, 49.

Ku, B.S. and H. Takeuchi, 1983, Effects of synthetic ergot derivatives on the two identifiable giant neurons, sensitive to dopamine, of *Achatina fulica* Férussac, Comp. Biochem. Physiol. 76C, 291.

Ku, B.S. and H. Takeuchi, 1985, Effects of catecholamines, monophenolamines and phenylamines on identifiable giant neurons of an African giant snail (*Achatina fulica* Férussac), Eur. J. Pharmacol. 114, 1.

Ku, B.S. and H. Takeuchi, 1986, Effects of catecholamine and monophenolamine agonists on identifiable giant neurones, sensitive to these amines, of an African giant snail (*Achatina fulica* Férussac), Eur. J. Pharmacol. 124, 21.

Lang, W.J. and O.L. Woodman, 1982, Comparison of the vasodilator action of dopamine and dopamine agonists in the renal and coronary beds of the dog, Br. J. Pharmacol. 77, 23.

Litchfield, Jr., J.T. and F. Wilcoxon, 1949, A simplified method of evaluating dose-effect experiments, J. Pharmacol. Exp. Ther. 96, 99.

Liu, G.J. and H. Takeuchi, 1993, Effects of cyclic AMP, cyclic GMP and $IP_3$ intracellularly injected into the identifiable *Achatina* giant neurones, Comp. Biochem. Physiol. 104C, 199.

Miyamoto, M., I. Yokoi, A. Sakai and H. Takeuchi, 1979, Excitatory effects of ergot alkaloids and their derivatives on the excitability of an identifiable giant neuron of the African giant snail (*Achatina fulica* Férussac). Arch. Int. Pharmacodyn. Ther. 241, 49.

Miyamoto, M., H. Tamura and H. Takeuchi, 1980, Inhibitory effects of ergot alkaloids and their derivatives on the excitability of an identifiable giant neurone of the African giant snail (*Achatina fulica* Férussac), Arch. Int. Pharmacodyn. Ther. 245, 56.

Molloy, A.G. and J.L. Waddington, 1985, The enantiomers of SK&F 83566, a new selective D-1 dopamine antagonist, stereospecifically block stereotyped behaviour induced by apomorphine and by the selective D-2 agonist RU 24213, Eur. J. Pharmacol. 116, 183.

Momiyama, T., N. Todo and M. Sasa, 1993, A mechanism underlying dopamine $D_1$ and $D_2$ receptor-mediated inhibition of dopaminergic neurones in the ventral tegmental area in vitro, Br. J. Pharmacol. 109, 933.

O'Boyle, K. and J.L. Waddington, 1985, Identification of the enantiomers of SK&F 83566 as specific and stereoselective antagonists at the striatal $D_1$ dopamine receptor: comparisons with the $D_2$ enantioselectivity of Ro 22-1319, Eur. J. Pharmacol. 106, 219.

Ohlstein, E.H., B. Zabko-Potapovich and B.A. Berkowitz, 1984, Studies on vascular dopamine receptors with dopamine receptor agonist: SK&F 82526, J. Pharmacol. Exp. Ther. 229, 433.

Okamoto, H., K. Takahashi and M. Yoshii, 1976, Membrane cur-

rents of the tunicate egg under the voltage-clamp condition, J. Physiol. (London) 254, 607.

Osborne, N.N., 1977, Adenosine 3',5'-monophosphate in snail (*Helix pomatia*) nervous system: analysis of dopamine receptors, Experientia 33, 917.

Setler, P.E., H.M. Sarau, C.L. Zirkle and H.L. Saunders, 1978, The central effects of a novel dopamine agonist, Eur. J. Pharmacol. 50, 419.

Sibley, D.R., S.E. Leff and I. Creese, 1982, Interactions of novel dopaminergic ligands with D-1 and D-2 dopamine receptors, Life Sci. 31, 637.

Sokoloff, P., B. Giros, M.-P. Martres, M.-L. Bouthenet and J.-C. Schwartz, 1990, Molecular cloning and characterization of a novel dopamine receptor ($D_3$) as a target for neuroleptics, Nature 347, 146.

Stoof, J.C., T.A. De Vlieger and J.C. Lodder, 1985, Opposing roles for D-1 and D-2 dopamine receptors in regulating the excitability of growth hormone-producing cells in the snail *Lymnaea stagnalis*, Eur. J. Pharmacol. 106, 431.

Sunahara, R.K., H.-C. Guan, B.F. O'Dowd, P. Seeman, L.G. Laurier, G. Ng, S.R. George, J. Torchia, H.H.M. Van Tol and H.B. Niznik, 1991, Cloning of the gene for a human dopamine $D_5$ receptor with higher affinity for dopamine than $D_1$, Nature 350, 614.

Svensson, K., A.M. Johansson, T. Magnusson and A. Carlsson, 1986, (+)-AJ76 and (+)-UH 232: central stimulants acting as preferential dopamine autoreceptor antagonists, Naunyn-Schmied. Arch. Pharmacol. 334, 234.

Takeuchi, H., T. Morimasa, M. Kohsaka, J. Kobayashi and F. Morii, 1973, Concentrations des ions inorganiques dans l'hémolymphe de l'Escargot géant africain (*Achatina fulica* Férussac) selon l'état de nutrition, C.R. Séanc. Soc. Biol. 167, 598.

Takeuchi, H., H.P. Boyles, B.S. Ku and K. Isobe, 1985a, Neurotransmetteurs des neurones géants identifiables chez l'Escargot géant africain, *Achatina fulica* Férussac. III. Les ganglions pédieux et les ganglions cérébraux, C.R. Séanc. Soc. Biol. 179, 769.

Takeuchi, H., B.S. Ku, T. Matsuoka, K. Watanabe and N. Yamamoto, 1985b, Neurotransmetteurs des neurones géants chez l'Escargot géant africain, *Achatina fulica* Férussac. II. Le ganglion viscéral, C.R. Séanc. Soc. Biol. 179, 761.

Takeuchi, H., B.S. Ku, T. Matsuoka, K. Watanabe, N. Yamamoto and K. Funase, 1985c, Neurotransmetteurs des neurones géants chez l'Escargot géant africain, *Achatina fulica* Férussac. I. Les ganglions pariétaux. C.R. Séanc. Soc. Biol. 179, 752.

Takeuchi, H., B.S. Ku, K. Watanabe, T. Matsuoka, K. Funase, X.P. Sun, A. Yongsiri, K.H. Kim and P.N. Li, 1987, Identification and pharmacological characteristics of giant neurones of an African giant snail (*Achatina fulica* Férussac), in: Neurobiology Molluscan Models, eds. H.H. Boer, W.P.M. Geraerts and J. Joosse (North-Holland Publishing Co., Amsterdam) p. 100.

Takeuchi, H., T. Matsuoka and K.H. Kim, 1988, Neurotransmetteurs des neurones géants chez l'Escargot géant africain, *Achatina fulica* Férussac. IV. Résultats complémentaires, C.R. Séanc. Soc. Biol. 182, 425.

Trabucchi, M., R. Longoni, P. Fresia and P.F. Spano, 1975, Sulpiride: a study of the effects on dopamine receptors in rat neostriatum and limbic forebrain, Life Sci. 17, 1551.

Van Tol, H.H.M., J.R. Bunzow, H.-C. Guan, R.K. Sunahara, P. Seeman, H.B. Niznik and O. Civelli, 1991, Cloning of the gene for a human dopamine $D_4$ receptor with high affinity for the antipsychotic clozapine, Nature 350, 610.

Waters, N., S. Lagerkvist, L. Lofberg, M. Piercey and A. Carlsson, 1993, The dopamine $D_3$ receptor and autoreceptor preferring antagonists (+)-AJ76 and (+)-UH232; a microdialysis study, Eur. J. Pharmacol. 242, 151.

Werkman, T.R., J.C. Lodder, T.A. De Vlieger and J.C. Stoof, 1987, Further pharmacological characterization of a D-2-like dopamine receptor on growth hormone producing cells in *Lymnaea stagnalis*, Eur. J. Pharmacol. 139, 155.

Yongsiri, A., T. Goto, N. Yamamoto, Y. Araki, H. Takeuchi and M. Namba, 1986, Axonal pathways of the four giant neurons identified in the cerebral ganglia of an African giant snail (*Achatina fulica* Férussac), Comp. Biochem. Physiol. 85A, 663.