Sex Differences in $[^3H]$Nitrendipine Binding and Effects of Sex Steroid Hormones in Rat Cardiac and Cerebral Membranes

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Abstract—The sex differences and regulation by sex steroid hormones in calcium channels were studied by using $[^3H]$nitrendipine binding to cardiac and cerebral membranes in 15-week old spontaneously hypertensive rats (SHRs). The maximal number of binding sites ($B_{max}$) in the hippocampus of female SHRs increased by 24.1% over that in male SHRs. In the females, the $B_{max}$ values in the cardiac, striatal, thalamic and hippocampal membranes from ovariectomized SHRs decreased by 34.7, 29.9, 29.3 and 26.9%, respectively, compared to normal SHRs. This phenomenon, except for the hippocampus, was inhibited by estradiol but not by testosterone. In the male, the $B_{max}$ values in cardiac and cerebral membranes showed almost no changes after orchidectomy or treatment with estradiol or testosterone. After gonadectomy, the $B_{max}$ values in the cardiac, striatal and thalamic membranes of females decreased by 30.2, 33.0 and 35.6%, respectively, compared to those in males. The changes in apparent dissociation constant ($K_D$) values were less remarkable than those in the $B_{max}$ values. These findings suggest that sex differences exist in the calcium channels of the heart, striatum, thalamus and hippocampus, and they suggest that estradiol, but not testosterone, may play a part in the regulation of the calcium channels in female SHRs.

It is well-known that sex differences exist in structures; e.g., in the size of specific brain regions, in dendritic and axonal branching patterns, and in the distribution of synapses (1), receptors (2–5), catecholamine contents (6) and neurotransmitter systems (7–9).

It is also well-known that calcium plays an important role in a variety of functions in both neuronal and non-neuronal tissues. In neuronal tissue, calcium influx into presynaptic nerve endings initiates a series of events leading to neurotransmitter release, whereas in the heart and smooth muscles, calcium couples excitation to contraction. The entry of calcium into these tissues appears to be mediated by a voltage-sensitive calcium channel (10, 11). Compounds termed “organic calcium channel antagonists” are reported to inhibit voltage-sensitive calcium channels (12, 13). Therefore, some calcium channel antagonists at certain concentrations can cause smooth muscle relaxation, negative inotropic and chronotropic effects or a decrease in neurotransmitter release. Although calcium channels play an important role in cell functions, no reports have been published about sex differences in calcium channels and their regulation by sex steroid hormones.

Recently, the binding properties of a calcium channel antagonist, $[^3H]$nitrendipine, a derivative of dihydropyridine, have been investigated in homogenates of the guinea-pig and rat cerebral cortex, heart and ileum (14, 15), and $[^3H]$nitrendipine has been used as a chemical probe for the chemical (16, 17) and biological (18–20) characterization of calcium channels.

In this study, we have attempted to characterize the sex differences of calcium channels, using $[^3H]$nitrendipine binding in the cardiac and cerebral membranes, and to examine the effects of sex steroid hormones on calcium channels in gonadectomized rats.
Materials and Methods

Animals: Male and female WKYs and SHRs aged 4 and 15 weeks were used. Male and female SHRs aged 4 weeks were anaesthetized with pentobarbitone (30 mg/kg, i.p.) and then bilaterally orchidectomized and/or ovariectomized through a scrotal and/or ventral route, respectively. Then the rats were maintained under ordinary environmental conditions for 15 weeks of age and were divided into 3 groups of male rats and 3 groups of female rats. The orchidectomy (Orchi) and/or ovariectomy (Ovari) groups consisted of orchidectomized and/or ovariectomized rats without hormonal treatment. The remaining 2 groups of male and female rats were alternatively given (s.c.) estradiol benzoate (estradiol) or testosterone benzoate (testosterone) dissolved in sesame oil in a dose of 250 μg/kg or 20 mg/kg, respectively, from 3 to 15 weeks of age.

Tissue preparation for binding assay: The heart and brain were quickly excised from untreated (control) male and female WKYs and SHRs (n=4, each), from gonadectomized male and female SHRs (n=6, each), and from gonadectomized male and female SHRs treated with estradiol and/or testosterone (n=5, each) aged 15 weeks, respectively. The hearts were homogenized in a Polytron P-10 using three 10 sec bursts in 10 volumes of 50 mM Tris-HCl buffer solution (pH 7.4) in ice water. The resulting homogenates were centrifuged at 1,000 x g for 10 min. The supernatants were centrifuged at 105,000 x g for 60 min. After the supernatants had been decanted, the pellets (crude membrane fractions) were resuspended in 50 mM Tris-HCl buffer solution (pH 7.4) to obtain a final protein concentration of 100 μg/2 ml and were used for the binding assay.

Assay of [3H]nitrendipine binding: A 2 ml aliquot of the thus prepared suspension was incubated with 20–800 pM [3H]nitrendipine in triplicate or duplicate for 90 min at 25°C. The mixture was filtered under vacuum through a Whatman GF/C glass fiber filter and washed with three 3 ml aliquots of 50 mM Tris-HCl buffer solution (pH 7.4). The trapped radioactivity was measured by liquid scintillation spectrophotometry (Packard, 300C type). Specific binding was defined as the difference between the total binding and binding in the presence of 1 μM nifedipine. The properties of [3H]nitrendipine binding were analyzed by the method of Scatchard (21).

Drugs: [3H]Nitrendipine (77.4 Ci/mmol) was obtained from the Japan Radioisotope Association (New England Nuclear, Boston, U.S.A.). Nifedipine was supplied by Bayer Yakuhin Co., Ltd., Osaka, Japan. 17β-Estradiol 3-benzoate (estradiol), testosterone benzoate (testosterone) and Tris-hydroxy-methylaminomethane (Tris) were obtained from Sigma Chemical Co., U.S.A. All other chemicals were of reagent grade.

Other methods: Protein was measured by the method of Lowry et al. (22) using bovine serum albumin as a standard. The results were expressed as the mean±standard error for each group. The significance of differences was examined by means of Student’s t-test.

Results

Properties of [3H]nitrendipine binding: The specific [3H]nitrendipine binding to crude membranes prepared from the heart and various areas of the brain of WKYs and SHRs aged 15 weeks was saturated monophasically (Fig. 1A–H). Using 20–800 pM [3H]nitrendipine yielded apparent dissociation constants (Kd) of 0.090 to 0.186 nM in the heart, striatum, thalamus and hippocampus for male and female WKYs and SHRs. Scatchard...
analysis revealed a single population with a Hill coefficient of 0.94-1.06 and showed a maximal number of binding sites (Bmax) of 57 to 229 fmol/mg protein in the heart, striatum, thalamus and hippocampus for male and female WKYs and SHRs (Fig. 2 A-H). Similar data were obtained from crude membrane preparations of gonadectomized male and female SHRs and from those of gonadectomized male and female SHRs treated with estradiol and/or testosterone (data not shown). The Bmax values of [3H]-nitrendipine binding sites in the striatum, thalamus and hippocampus in the male and female SHRs and in the heart in the female SHRs significantly increased over those in the male and female WKYs and in the female WKYs, but the Kp values differed very little between the WKY and SHR groups (Table 1).

Properties of [3H]-nitrendipine binding to the cardiac membranes: The Kp values in the female control group showed a significant increase over those in the male control group, but the Bmax values differed very little between the male and female control groups (Fig. 3). In the groups treated with gonadectomy, the Bmax values in the Ovari group showed a significant (P<0.001) decrease of 34.7% over those in the female control group and a decrease of 30.2% over those in the Orchi group, but the Kp and Bmax values differed very little between the Orchi group and the male control group. In the males, the Kp values in the estradiol and/or testosterone groups and the control and/or Orchi groups. In the females, the Kp values in the estradiol and testosterone groups showed significant increases over the respective values in the Ovari group. The Bmax values in the estradiol group showed a significant of 40.4% over those in the Ovari group, but an insignificant decrease of 8.3% over those in the control group. The Bmax values in the testosterone group showed an
insignificant increase of 17.7% over those in the Ovari group, but a significant decrease of 23.1% over those in the control group.

Table 1. Properties of [3H]nitrendipine binding to cardiac and cerebral membranes from male and female WKY and SHR aged 15 weeks

|          | Male                        | Female                       |
|----------|-----------------------------|------------------------------|
|          | K_D (nM)                    | B_max (fmol/mg protein)     | K_D (nM)                    | B_max (fmol/mg protein) |
| Heart    |                             |                              |                             |                         |
| WKY      | 0.093±0.0054                | 210±5.4                      | 0.136±0.0100^c              | 171±11.9^d              |
| SHR      | 0.090±0.0061                | 229±10.5                     | 0.108±0.0004^a              | 216±12.5^a              |
| Striatum |                             |                              |                             |                         |
| WKY      | 0.153±0.0033                | 65±1.5                       | 0.125±0.0072^d              | 63±3.4                  |
| SHR      | 0.183±0.0121                | 91±1.4^c                     | 0.133±0.0107^d              | 87±2.6^b                |
| Thalamus |                             |                              |                             |                         |
| WKY      | 0.165±0.0274                | 61±2.7                       | 0.167±0.0594                | 57±3.1                  |
| SHR      | 0.155±0.0067                | 81±2.8^a                     | 0.186±0.0180                | 82±3.8^b                |
| Hippocampus |                             |                              |                             |                         |
| WKY      | 0.132±0.0043                | 108±3.8                      | 0.147±0.0216                | 126±2.9^e              |
| SHR      | 0.143±0.0121                | 141±3.2^c                    | 0.147±0.0142                | 175±4.9^cv             |

Fig. 3. Properties of [3H]nitrendipine binding to cardiac membranes from untreated (control) SHRs, from gonadectomized SHRs and from gonadectomized SHRs treated with estradiol and/or testosterone. The properties of [3H]nitrendipine binding were analyzed by the method of Scatchard. Each experiment was performed four times. The values express the mean±S.E. ^aP<0.05, ^bP<0.01 and ^cP<0.001: significantly different from male or female WKY, respectively. ^dP<0.05 and ^eP<0.01: significantly different from male WKY or SHR, respectively.

Properties of [3H]nitrendipine binding in the striatal membranes: The K_D values in the female control group showed a significant decrease over those in the male control group, but the B_max values differed very little between
In the groups treated with gonadectomy, the $B_{\text{max}}$ values in the Ovari group showed a significant decrease of 29.9% over those in the female control group and a significant ($P<0.01$) decrease of 33.0% over those in the Orchi group, but the $K_D$ and $B_{\text{max}}$ values differed very little between the Orchi group and the male control group. In the males, the $K_D$ and $B_{\text{max}}$ values differed very little between the estradiol and/or testosterone groups and the control and/or Orchi groups. In the females, the $B_{\text{max}}$ values in the estradiol group showed an insignificant increase of 13.1%, but those in the testosterone group showed an insignificant decrease of 6.7% over those in the Ovari group. Decreases of 20.7 and 34.5%, respectively, over those in the control group were significant. The $K_D$ values differed very little between the estradiol and/or testosterone groups and the control and/or Ovari groups.

**Properties of [$^3$H]nitrendipine binding in the thalamic membranes:** The $K_D$ and $B_{\text{max}}$ values differed very little between the male and female control groups (Fig. 5). In the groups treated with gonadectomy, the $B_{\text{max}}$ values in the Orchi group showed a significant increase of 11.1% over those in the male control group, while the $K_D$ values differed very little between the Orchi group and the male control group. The $B_{\text{max}}$ values in the Ovari group showed a significant decrease of 29.3% over those in the female control group, but the $K_D$ values differed very little between the Orchi group and the male control group. Furthermore, $B_{\text{max}}$ values in the Ovari group showed a significant increase of 13.1%, but those in the testosterone group showed a significant decrease of 6.7% over those in the control group. In the males, the $K_D$ values in the estradiol group showed a significant decrease over those in the control group, while the $B_{\text{max}}$ values differed very little between the estradiol group and the control or Orchi groups. In the testosterone group, the $K_D$ values showed a significant decrease over those in the Orchi group, and the $B_{\text{max}}$ values showed a significant decrease of 25.6% over those in the Orchi group, but showed an insignificant decrease of 17.3% over those in the control group. In the females, the $B_{\text{max}}$ values in the estradiol group showed an insignificant increase of 32.8% over those in the Ovari group, but a significant decrease of 23.2% over those in the control group. The $K_D$ values differed very little between the estradiol and/or testosterone-
one groups and the Ovari and control groups.

Properties of $[^{3}H]$nitrendipine binding in the hippocampal membranes: The $B_{\text{max}}$ values in the female control group showed a significant increase of 24.1% over those in the male control group, but the $K_D$ values differed very little between the male and the female control groups (Fig. 6). In the groups treated with gonadectomy, the $K_D$ values in the Orchi group showed a significant decrease over those in the male control group, but the $B_{\text{max}}$ values differed very little between the male and the Orchi group. In the Ovari group, the $B_{\text{max}}$ values showed a significant decrease of 26.9% over those in the female control group, but the $K_D$ values dif-
fered very little between the Ovari group and the female control group. Also, the $K_D$ and $B_{\text{max}}$ values differed very little between the Orchi group and the Ovari group. In the males, the $B_{\text{max}}$ values differed very little between the estradiol and/or testosterone groups and the Orchi and/or control groups, but the $K_D$ values in the estradiol group showed a significant decrease over those in the control group. In the females, the $K_D$ and $B_{\text{max}}$ values differed very little between the estradiol group and the Ovari group, but the $B_{\text{max}}$ values in the estradiol group showed a significant decrease of 26.9% over those in the control group. In the Testosterone group, the $B_{\text{max}}$ values showed an insignificant decrease of 10.9% over those in the Ovari group, but a significant decrease of 43.9% over those in the control groups, while the $K_D$ values showed a significant decrease over those in the control group.

Discussion

The specific bindings of $[3\text{H}]$nitrendipine to crude cardiac and cerebral membranes from WKYs and SHRs aged 15 weeks were saturable (Fig. 1) with high affinity and $K_D$ values of 0.090–0.186 nM (Fig. 2). The observed $K_D$ and $B_{\text{max}}$ values for cardiac and cerebral membranes agreed with those reported by Ehlert et al. (15), DePover et al. (23), Gould et al. (24) and Ishii et al. (25).

Our research (25) showed that the $B_{\text{max}}$ values of $[3\text{H}]$nitrendipine binding sites in the striatum, thalamus and hippocampus in the male SHRs significantly increased over those in the male WKYs. In this study, increases of $[3\text{H}]$nitrendipine binding sites in the striatum, thalamus and hippocampus in the female SHRs were also observed. Furthermore, the $B_{\text{max}}$ values of $[3\text{H}]$nitrendipine binding sites in the heart, striatum and thalamus of the Ovari group significantly decreased over those in the untreated control group (Figs. 3–6). The $B_{\text{max}}$ values in the heart, striatum and thalamus of the Ovari group significantly decreased over those in the Orchi group, while the $B_{\text{max}}$ values in the hippocampus differed very little between the Ovari and Orchi groups. Furthermore, the $K_D$ values in the males, except for the hippocampus, differed very little between the gonadectomy and untreated control groups. Those data suggest that the differences between males and females in the number of $[3\text{H}]$nitrendipine binding sites observed in the heart, striatum, thalamus and hippocampus of SHRs were sex differences and that sex steroid hormones may elicit an increase in the number of $[3\text{H}]$nitrendipine binding sites in the female SHRs.

When the SHRs were treated with gonadectomy, the $B_{\text{max}}$ values in the heart, striatum, thalamus and hippocampus of the female SHRs significantly decreased over those in the untreated female control group, but in the male SHRs, except for the thalamus, the $B_{\text{max}}$ values were not significantly different from those in the untreated male control group (Figs. 3–6). The $B_{\text{max}}$ values in the heart, striatum and thalamus of the Ovari group significantly decreased over those in the Orchi group, while the $B_{\text{max}}$ values in the hippocampus differed very little between the Ovari and Orchi groups. Furthermore, the $K_D$ values in the males, except for the hippocampus, differed very little between the gonadectomy and untreated control groups. Those data suggest that the differences between males and females in the number of $[3\text{H}]$nitrendipine binding sites observed in the heart, striatum, thalamus and hippocampus of SHRs were sex differences and that sex steroid hormones may elicit an increase in the number of $[3\text{H}]$nitrendipine binding sites in the female SHRs.

When the ovariectomized SHRs were given estradiol, the $B_{\text{max}}$ values in the heart, striatum and thalamus, but not in the hippocampus, increased over those in the Ovari group, and had a tendency to return to those of the control group (Figs. 3–6), whereas in the male SHRs, estradiol did not influence the $B_{\text{max}}$ values of binding sites in any of the tissues. Changes in the $K_D$ values in both male and female SHRs were less remarkable than those in the $B_{\text{max}}$ values. These data suggest that estradiol may play a part in the increase in the number of $[3\text{H}]$nitrendipine binding sites in the heart, striatum and thalamus of female SHRs, and they suggest that in the male SHRs, estrogen receptors related to regulation of $[3\text{H}]$nitrendipine binding sites mostly exist in the heart, striatum, thalamus and hippocampus. However, the role of estradiol in the hippocampus of female SHRs could not be specified. On the other hand, testosterone in both male and female SHRs hardly affected the $K_D$ and $B_{\text{max}}$ values of binding sites. However, we have also reported (26) that the $B_{\text{max}}$ values of $[3\text{H}]$nitrendipine binding in crude seminal vesicle membranes...
from orchidectomized rats increased from 49±2.4 to 91±6.9 fmol/mg protein after treatment with testosterone, and that the Kᵦ values in the testosterone treated group differed very little from those in the orchidectomized group. Therefore, it may be concluded that the action(s) of testosterone display tissue specificity depending on the differences in the numbers of androgen receptors, and that [³H]nitrendipine binding sites in the heart and brain of male and female rats are not affected by testosterone.

Dihydropyridine calcium channel antagonists and agonists have been used extensively as probes of the calcium channel (27, 28). However, these drugs usually have little or no influence on Ca²⁺-influx and transmitter release induced by depolarization of presynaptic terminals (29). On the other hand, Middlemiss and Spedding (30) reported that Bay K 8644, a dihydropyridine calcium channel agonist, could augment the K⁺-stimulated release of serotonin from frontal cortex slices and that these effects could be antagonized by a low concentration of calcium channel antagonists. Turner and Goldin (31) also reported that depolarization-stimulated synaptosomal ⁴⁶Ca²⁺ uptake in the absence of external Na⁺ was potently blocked by dihydropyridines. Recently, Cruz et al. (32) reported that there are four types (N, T, L, and Lₐ) of calcium channels in chick tissues when they used [¹²⁵I]-ω-conotoxin, a class of calcium channel antagonist from fish hunting marine snails, and [³H]nitrendipine binding studies indicated that there are more ω-conotoxin binding sites (N+Lₐ) than dihydropyridine binding sites (Lₐ alone) in the brain. These reports suggest that [³H]nitrendipine binding sites may be thought of as labeling voltage-sensitive calcium channels themselves or their components in the neuronal tissues.

In conclusion, sex differences in the heart, striatum, thalamus and hippocampus exist in terms of the number of [³H]nitrendipine binding sites reflected in calcium channels resembling voltage-sensitive channels or their components. Furthermore, estradiol, but not testosterone, may play a part in the regulation of these calcium channels in female SHRs.

References

1 Raisman, G. and Field, P.M.: Sexual dimorphism in the preoptic area of the rat. Science 173, 731–733 (1971)
2 Avissar, S., Egozi, Y. and Sokolovsky, M.: Studies of muscarinic receptors in mouse and rat hypothalamus: a comparison of sex and cyclical differences. Neuroendocrinology 32, 295–302 (1981)
3 Orensanz, L.M., Guillamon, A., Ambrosio, E., Segovia, S. and Azuara, M.C.: Sex differences in apha-adrenergic receptors in the rat brain. Neurosci. Lett. 30, 275–278 (1982)
4 De Vries, G.J., Best, W. and Sluiter, A.A.: The influence of gonadal steroids on a sex difference in the vasopressinergic innervation of the brain. Dev. Brain Res. 8, 377–380 (1983)
5 Fischette, C.T., Biegon, A. and McEwen, B.S.: Sex differences in serotonin 1 receptor binding in rat brain. Science 222, 333–335 (1983)
6 Crowley, W., O’Donohue, T. and Jacobowitz, W.: Sex differences in catecholamine contents indescrete brain nuclei of the rat: effect of neonatal castration of testosterone treatment. Acta Endocrinol. (Copenh.) 29, 20–28 (1978)
7 Gordon, J.H. and Sheffeler, M.K.: Regional catecholamine content in the rat brain: sex difference and correlation with motor activity. Neuropsychopharmacology 13, 129–137 (1974)
8 Becker, J.B. and Ramirez, V.D.: Sex differences in the amphetamine stimulated release of catecholamines from rat striatal tissue in vitro. Brain Res. 204, 361–372 (1981)
9 Luine, V.N. and McEwen, B.S.: Sex differences in cholinergic enzymes of diagonal band nuclei in the rat preoptic area. Neuroendocrinology 36, 475–482 (1983)
10 Hagiwara, S. and Byerly, L.: Calcium channel. Annu. Rev. Neurosci. 4, 69–125 (1981)
11 Cauvin, C., Loutzenhiser, R. and Van Breemen, C.: Mechanisms of calcium antagonist-induced vasodilation. Annu. Rev. Pharmacol. Toxicol. 23, 375–396 (1983)
12 Fleckenstein, A.: Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. Annu. Rev. Pharmacol. Toxicol. 17, 149–166 (1977)
13 Karaki, H., Nakagawa, H. and Uraeawa, N.: Comparative effects of verapamil and sodium nitroprusside on contraction and ⁴⁶Ca uptake in the smooth muscle of rabbit aorta, rat aorta and guinea-pig taenia coli. Br. J. Pharmacol. 81, 393–400 (1984)
14 Bellemann, P., Ferry, D., Lübbeck, F. and Glossmann, H.: [³H]-Nitrendipine, a potent
15 Ehlert, G.J., Roeske, W.R., Itoga, E. and Yamamura, H.I.: The binding of [3H]nitrendipine to receptors for calcium channel antagonists in the heart, cerebral cortex, and ileum of rats. Life Sci. 30, 2191–2202 (1982)

16 Curtis, B.M. and Catterall, W.A.: Purification of the calcium antagonist receptor of the voltage-sensitive calcium channel from skeletal muscle transverse tubules. Biochemistry 23, 2113–2118 (1984)

17 Venter, J.C., Fraser, C.M., Schaber, J.S., Jung, C.Y., Bolger, G. and Triggie, D.J.: Molecular properties of the slow inward calcium channel: molecular weight determinations by radiation inactivation and covalent affinity labeling. J. Biol. Chem. 258, 9344–9348 (1984)

18 Ishii, K., Kano, T., Kurobe, Y. and Ando, J.: Binding of [3H]nitrendipine to heart and brain membranes from normotensive and spontaneously hypertensive rats. Eur. J. Pharmacol. 88, 277–278 (1983)

19 Schmid, A., Kazazoglou, T., Renaud, J.-F. and Lazdunski, M.: Comparative changes of levels of nitrendipine Ca²⁺ channels, of tetrodotoxin-sensitive Na⁺ channels and of ouabain-sensitive (Na⁺+K⁺)-ATPase following denervation of rat and chick skeletal muscle. FEBS Lett. 172, 114–118 (1984)

20 Saito, K., Ishii, K., Fujita, N., Nakahiro, M. and Inoki, R.: Selective enhancement in striatal [3H]-nitrendipine binding following chronic treatment with morphine. Neurochem. Int. 7, 1033–1036 (1985)

21 Scatchard, G.: The attractions of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. 51, 660–672 (1949)

22 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275 (1951)

23 DePover, A., Matlab, M.A., Lee, S.W., Dubé, G.P., Grupp, I.L., Grupp, G. and Schwartz, A.: Specific binding [3H]nitrendipine to membranes from coronary arteries and heart in relation to pharmacological effects. Paradoxical stimulation by diidiazem. Biochem. Biophys. Res. Commun. 108, 110–117 (1982)

24 Gould, R.J., Murphy, K.M.M. and Snyder, S.H.: Tissue heterogeneity of calcium channel antagonist binding sites labeled by [3H]nitrendipine. Mol. Pharmacol. 25, 235–241 (1984)

25 Ishii, K., Kano, T., Ando, J. and Yoshida, H.: Binding of [3H]nitrendipine to cardiac and cerebral membranes from normotensive and renal, deoxycorticosterone/NaCl and spontaneously hypertensive rats. Eur. J. Pharmacol. 123, 271–278 (1986)

26 Ishii, K., Kano, T. and Ando, J.: Calcium channel and mechanical reactivity of testosterone-treated rat seminal vesicle. Japan. J. Fet. Ster. (in press)

27 Glossmann, H., Ferry, D.R., Lübbeke, F., Mewes, R. and Hofmann, F.: Calcium channels: direct identification with radioligand binding studies. TIPS 3, 431–437 (1982)

28 Tower, R. and Schramm, M.: Recent advances in the pharmacology of the calcium channel. TIPS 5, 111–113 (1984)

29 Miller, R.J.: How many types of calcium channels exist in neurones? TIPS 8, 45–47 (1985)

30 Middlemiss, D.N. and Spedding, M.: A functional correlate for the dihydropyridine binding site in the rat brain. Nature 314, 94–96 (1985)

31 Turner, T.J. and Goldin, S.M.: Calcium channels in rat brain synaptosomes: Identification and pharmacological characterization. High affinity blockade by organic Ca²⁺ channel blockers. J. Neurosci. 5, 841–849 (1985)

32 Cruz, L.J., Johnson, D.S. and Olivera, B.M.: Characterization of the α-conotoxin target. Evidence for tissue-specific heterogeneity in calcium channel types. Biochemistry 26, 820–824 (1987)