Effects of Ca\(^{2+}\) channel antagonists on acetylcholine and catecholamine releases in the in vivo rat adrenal medulla

Tsuyoshi Akiyama, Toji Yamazaki, Hideo Mori, and Kenji Sunagawa

1Department of Cardiovascular Dynamics, National Cardiovascular Center Research Institute, Osaka 565–8565, Japan
2Department of Cardiac Physiology, National Cardiovascular Center, Suita, Osaka 565–8565, Japan

Submitted 20 October 2003; accepted in final form 13 March 2004

Am J Physiol Regul Integr Comp Physiol 287: R161–R166, 2004. First published March 18, 2004; 10.1152/ajpregu.00609.2003.—To elucidate the type of voltage-dependent Ca\(^{2+}\) channels controlling ACh and catecholamine releases in the in vivo adrenal medulla, we implanted microdialysis probes in the left adrenal medulla of anesthetized rats and investigated the effects of Ca\(^{2+}\) channel antagonists on ACh, norepinephrine, and epinephrine releases induced by nerve stimulation. The dialysis probes were perfused with Ringer solution containing a cholinesterase inhibitor, neostigmine. The left splanchnic nerves were electrically stimulated at 2 and 4 Hz before and after intravenous administration of Ca\(^{2+}\) channel antagonists, ω-Conotoxin GVIA (an N-type Ca\(^{2+}\) channel antagonist, 10 μg/kg) inhibited ACh release at 2 and 4 Hz by ~40%, norepinephrine release at 4 Hz by ~50%, and epinephrine release at 2 and 4 Hz by ~45%. A fivefold higher dose of ω-conotoxin GVIA (50 μg/kg) did not further inhibit these releases. ω-Conotoxin MVIIC (a P/Q-type Ca\(^{2+}\) channel antagonist, 50 μg/kg) inhibited ACh and epinephrine releases at 4 Hz by ~30%. Combined ω-conotoxin GVIA (50 μg/kg) and MVIIC (250 μg/kg) inhibited ACh and epinephrine releases at 4 Hz by ~70% and norepinephrine and epinephrine releases at 2 and 4 Hz by ~80%. Nifedipine (an L-type Ca\(^{2+}\) channel antagonist, 300 and 900 μg/kg) did not change ACh release at 2 and 4 Hz; however, nifedipine (300 μg/kg) inhibited epinephrine release at 4 Hz by 20%, and nifedipine (900 μg/kg) inhibited norepinephrine and epinephrine releases at 4 Hz by 30%. In conclusion, both N- and P/Q-type Ca\(^{2+}\) channels control ACh release on preganglionic splanchnic nerve endings while L-type Ca\(^{2+}\) channels do not. L-type Ca\(^{2+}\) channels are involved in norepinephrine and epinephrine releases on chromaffin cells.

Anesthetized rats; microdialysis; norepinephrine; epinephrine; preganglionic autonomic nerve endings

Ca\(^{2+}\) influx through the voltage-dependent Ca\(^{2+}\) channels induces the release of transmitters from neuronal or secretory cells by initiating exocytosis from vesicles. Voltage-dependent Ca\(^{2+}\) channels have been classified into L-, N-, P-, Q-, R-, and T-types (12, 25, 30). To better understand the mechanism controlling the release of transmitters, it is important to determine the type of Ca\(^{2+}\) channels involved in the release of the transmitters on neuronal or secretory cells.

In the in vivo adrenal medulla, catecholamine release is controlled by central sympathetic neurons through preganglionic splanchnic nerves. Splanchnic nerve endings make synaptic-like contacts with chromaffin cells (9). ACh released from splanchnic nerve endings consequently evokes catecholamine release from chromaffin cells by activation of cholinergic receptors. Thus, in vivo catecholamine release requires Ca\(^{2+}\) influx through the voltage-dependent Ca\(^{2+}\) channels at two different sites in the adrenal medulla: splanchnic nerve endings and chromaffin cells. Numerous studies have investigated the nature of Ca\(^{2+}\) channels controlling transmitter release from postganglionic autonomic nerve endings (8, 11, 32, 33, 36, 37). Little information is, however, available on the type of Ca\(^{2+}\) channels controlling the ACh release from preganglionic autonomic nerve endings including splanchnic nerve endings. Moreover, although the types of Ca\(^{2+}\) channels controlling catecholamine release have been investigated using isolated chromaffin cells in various species (5, 6, 13, 16, 21, 23, 24), it remains unknown whether endogenous ACh induces Ca\(^{2+}\) influx through the same types of Ca\(^{2+}\) channels on chromaffin cells.

We have recently developed a dialysis technique to simultaneously monitor ACh and catecholamine releases in the in vivo adrenal medulla (2). This method makes it possible to characterize Ca\(^{2+}\) channels controlling ACh release from splanchnic nerve endings and catecholamine release from adrenal medulla in the in vivo state. In the present study, we applied the microdialysis technique to the adrenal medulla of anesthetized rats and investigated the effects of Ca\(^{2+}\) channel antagonists on dialysate ACh and catecholamine responses induced by the electrical stimulation of splanchnic nerves.

MATERIALS AND METHODS

Animal preparation. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85–23, revised 1996). Adult male Wistar rats weighing 380–450 g were anesthetized with pentobarbital sodium (50–55 mg/kg ip). A cervical midline incision was made to expose the trachea, which was then cannulated. The rats were ventilated with a constant-volume respirometer using room air mixed with oxygen. The left femoral artery and vein were cannulated for monitoring arterial blood pressure and administration of anesthetic, respectively. The level of anesthesia was maintained with a continuous intravenous infusion of pentobarbital sodium (15–25 mg·kg\(^{-1}\)·h\(^{-1}\) iv). Electrocardiogram was monitored for recording heart rate. A thermostat heating pad was used to keep the esophageal temperature within a range of 37–38°C. With the animal in the lateral position, the left adrenal gland and left splanchnic nerve were exposed by a subcostal flank incision, and the left splanchnic nerve was transected. Shielded bipolar stainless steel electrodes were applied to the distal end of the nerve, which was then stimulated with a digital stimulator (SEN-7203, Nihon Kohden) with a rectangular pulse (10 V and 1 ms in duration).
Dialysis technique. The materials of the dialysis probe were the same as those used in our previous dialysis experiments (1, 2). Briefly, each end of the dialysis fiber (0.31 mm OD, and 0.20 mm ID; PAN-1200 50,000 mol wt cutoff, Asahi Chemical) was inserted into the polyethylene tube (25-cm length, 0.5 mm OD, and 0.2 mm ID; SP-8) and glued. The length of the dialysis fiber exposed was 3 mm.

The left adrenal gland was gently lifted, and the dialysis probe was implanted in the medulla of the left adrenal gland along the long axis by using a fine guiding needle. The dialysis probe was perfused with Ringer solution containing a cholinesterase inhibitor, neostigmine (10 µM) and at a speed of 10 µl/min using a microinjection pump (CMA/100, Carnegie Medicin). Ringer solution with no buffer consisted of (in mM) 147.0 NaCl, 4.0 KCl, and 2.25 CaCl2. One sampling period was 2 min (1 sample volume = 20 µl). We started the protocols followed by a stabilization period of 3–4 h and sampled dialysate taking the dead space volume into account.

Dialysate ACh, norepinephrine (NE), and epinephrine (Epi) concentrations were measured as indexes of ACh and catecholamine releases in the adrenal medulla. Half of the dialysate sample was used for the measurement of ACh, and the remaining half for the measurement of NE and Epi. ACh and catecholamine assays were separately conducted using each high-performance liquid chromatography with electrochemical detection as previously described (3, 4).

Experimental design. The experiment was performed based on the previous experiment showing that dialysate ACh and catecholamine responses were reproducible on repetition of stimulation (2). The left splanchnic nerves were electrically stimulated for 2 min at 30-min intervals. Three dialysate samples were continuously collected per electrical stimulation: one before, one during, and one after stimulation. Stimulations at two different frequencies (2 and 4 Hz) were observed in dialysate before stimulation. Intravenous administration of Ca²⁺ channel antagonists did not affect these basal NE and Epi releases (Table 1). Dialysate NE and Epi concentrations increased by nerve stimulation and rapidly declined after the stimulation. Thus we subtracted the dialysate NE and Epi contents before stimulation from those during stimulation and expressed these values as indexes of NE and Epi releases induced by stimulation.

Effects of Ca²⁺ channel antagonists on heart rate and mean arterial pressure. ω-Conotoxin GVIA (10 µg/kg) decreased heart rate from 418 ± 9 to 328 ± 13 beats/min (P < 0.05) and mean arterial pressure from 115 ± 2 to 74 ± 2 mmHg (P < 0.05). ω-Conotoxin GVIA (50 µg/kg) did not further decrease heart rate and mean arterial pressure. ω-Conotoxin MVIIIC decreased heart rate from 408 ± 3 to 390 ± 5 beats/min (P < 0.05) but did not change mean arterial pressure. Combined ω-conotoxin GVIA and MVIIIC decreased heart rate from 415 ± 10 to 327 ± 4 beats/min (P < 0.05) and mean arterial pressure from 124 ± 2 to 57 ± 2 mmHg (P < 0.05). Nifedipine (300 µg/kg) decreased mean arterial pressure from 113 ± 4 to 86 ± 4 mmHg (P < 0.05) but did not change heart rate. Nifedipine (900 µg/kg) decreased mean arterial pressure from 124 ± 3 to 73 ± 2 mmHg (P < 0.05).

Effects of Ca²⁺ channel antagonists on ACh and catecholamine releases. ACh could not be detected in dialysate before or after stimulation. Thus we expressed dialysate ACh concentration during stimulation as an index of ACh release induced by stimulation. In contrast, substantial amounts of NE and Epi were observed in dialysate before stimulation. Intravenous administration of Ca²⁺ channel antagonists did not affect these basal NE and Epi releases (Table 1). Dialysate NE and Epi concentrations increased by nerve stimulation and rapidly declined after the stimulation. Thus we subtracted the dialysate NE and Epi contents before stimulation from those during stimulation and expressed these values as indexes of NE and Epi releases induced by stimulation.

Table 1. Basal dialysate NE and Epi concentrations before and after administration of Ca²⁺ channel antagonists

<table>
<thead>
<tr>
<th>NE, nM</th>
<th>Epi, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>ω-Conotoxin GVIA (10 and 50 µg/kg) (n = 12)</td>
<td>Before administration 4.9 ± 0.9 20.6 ± 2.9 After administration 3.8 ± 0.6 21.0 ± 2.6</td>
</tr>
<tr>
<td>ω-Conotoxin MVIIIC (50 µg/kg) (n = 6)</td>
<td>Before administration 4.1 ± 1.0 20.2 ± 2.6 After administration 4.6 ± 0.9 24.0 ± 3.5</td>
</tr>
<tr>
<td>ω-Conotoxin GVIA (50 µg/kg) + MVIIIC (250 µg/kg) (n = 6)</td>
<td>Before administration 4.4 ± 1.3 17.5 ± 3.8 After administration 3.1 ± 0.7 20.8 ± 2.4</td>
</tr>
<tr>
<td>Nifedipine (100 and 300 µg/kg) (n = 12)</td>
<td>Before administration 4.0 ± 0.6 17.4 ± 2.2 After administration 3.3 ± 0.9 17.2 ± 2.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. NE, norepinephrine; Epi, epinephrine.
further inhibit release. \(\omega\)-Conotoxin GVIA (50 \(\mu\)g/kg) significantly inhibited ACh release at 2 Hz from 6.5 ± 0.7 to 3.7 ± 0.5 nM, ACh release at 4 Hz from 12.6 ± 1.4 to 8.5 ± 0.8 nM, NE release at 4 Hz from 41 ± 6 to 24 ± 4 nM, Epi release at 2 Hz from 70 ± 10 to 40 ± 6 nM, and Epi release at 4 Hz from 170 ± 15 to 112 ± 10 nM (Fig. 1B).

**Effects of \(\omega\)-conotoxin MVIIC.** \(\omega\)-Conotoxin MVIIC (50 \(\mu\)g/kg) significantly inhibited ACh release at 4 Hz from 11.7 ± 2.5 to 8.5 ± 2.1 nM and Epi release at 4 Hz from 170 ± 38 to 129 ± 35 nM. Inhibitions of ACh and Epi releases at 2 Hz and NE release at either frequency were not statistically significant (Fig. 2).

**Effects of combined \(\omega\)-conotoxin GVIA and MVIIC.** Combined \(\omega\)-conotoxin GVIA (50 \(\mu\)g/kg) and MVIIC (250 \(\mu\)g/kg) significantly inhibited ACh release at 2 Hz from 6.7 ± 0.6 to 1.9 ± 0.3 nM, ACh release at 4 Hz from 12.1 ± 1.3 to 3.8 ± 0.6 nM, NE release at 2 Hz from 11.1 ± 1.1 to 1.2 ± 0.3 nM, NE release at 4 Hz from 36 ± 5 to 8 ± 2 nM, Epi release at 2 Hz from 88 ± 9 to 13 ± 3 nM, and Epi release at 4 Hz from 187 ± 20 to 49 ± 9 nM (Fig. 3).

**Effects of nifedipine.** Nifedipine (300 \(\mu\)g/kg) did not change ACh release at either frequency but significantly inhibited Epi release at 4 Hz from 172 ± 31 to 135 ± 23 nM. Inhibitions of Epi release at 2 Hz and NE release at either frequency were not statistically significant (Fig. 4A). A threefold higher dose of nifedipine (900 \(\mu\)g/kg) did not change ACh release but significantly inhibited Epi release at 4 Hz from 188 ± 24 to 128 ± 15 nM and NE release at 4 Hz from 33 ± 5 to 24 ± 4 nM. Inhibitions of NE and Epi releases at 2 Hz were not statistically significant (Fig. 4B).

**DISCUSSION**

Effects of \(\text{Ca}^{2+}\) channel antagonists on ACh release from splanchnic nerve endings. In the present study, \(\omega\)-conotoxin GVIA (10 \(\mu\)g/kg) inhibited ACh release at both 2 and 4 Hz by approximately 35–40%. A fivefold higher dose of \(\omega\)-conotoxin GVIA (50 \(\mu\)g/kg) did not further inhibit ACh release. \(\omega\)-Conotoxin MVIIC (50 \(\mu\)g/kg) inhibited ACh release at 4 Hz by ~30%. Combined \(\omega\)-conotoxin GVIA (50 \(\mu\)g/kg) and MVIIC (250 \(\mu\)g/kg) inhibited ACh release at both 2 and 4 Hz by ~70%. N- and P/Q-type \(\text{Ca}^{2+}\) channels could be present on the splanchnic nerve endings and be involved in ACh release. P/Q-type \(\text{Ca}^{2+}\) channels may play a role in ACh release at a high frequency of stimulation. ACh release response was resistant to nifedipine (300 and 900 \(\mu\)g/kg) at both 2 and 4 Hz. L-type \(\text{Ca}^{2+}\) channels could not be present on splanchnic nerve endings.
endings or not play a major role in ACh release. This is the first direct study to demonstrate the type of Ca\(^{2+}\) channels controlling ACh release from splanchnic nerve endings.

In isolated rat adrenal glands, catecholamine release induced by field stimulation is sensitive to P/Q-type Ca\(^{2+}\) channel antagonist, whereas that induced by exogenous ACh is insensitive (27). This indirect study suggested the involvement of P/Q-type Ca\(^{2+}\) channels in ACh release but failed to show the involvement of N-type Ca\(^{2+}\) channels. In isolated bovine adrenal glands, a direct measurement study showed that a reduction of the extracellular Ca\(^{2+}\) concentration inhibits \(^{3}\text{H}\)-labeled ACh release induced by field stimulation, but N- and L-type Ca\(^{2+}\) channel antagonists do not (28). Thus our findings are in part consistent with these direct and indirect studies but inconsistent as to the involvement of N-type Ca\(^{2+}\) channels.

This discrepancy might be ascribed to the experimental method. The contribution of Ca\(^{2+}\) channels may vary with the type of method used to evoke ACh release. In these studies, ACh release was evoked by electrical field stimulation of isolated adrenal glands, which is known to induce ACh release but not direct depolarization of chromaffin cells (34). In the present study, ACh release was evoked in the in vivo state by electrical stimulation of splanchnic nerves. The type of Ca\(^{2+}\) channels involved in ACh release may vary with the frequency, amplitude, or time period of stimulation. Actually, in the present study, we observed the involvement of P/Q-type Ca\(^{2+}\) channels at only high-frequency stimulation, while it has been reported in perfused rat adrenal glands that N-type Ca\(^{2+}\) channels are involved in the maintenance of catecholamine release in response to long splanchnic nerve stimulation (31). The time period of 2 min in the present study seems to be longer than those in earlier studies but could be within the physiological range. Moreover, the blocking action of \(\omega\)-conotoxin GVIA is time dependent as well as dose dependent and irreversible (8, 11, 32, 36). The maximum functional effect of \(\omega\)-conotoxin GVIA has been observed to be at least 15 min after administration. We evaluated the effect of \(\omega\)-conotoxin GVIA 30 min after intravenous administration, when heart rate and mean arterial pressure had already been stabilized. The evaluation early after administration might lead to underestimation of the inhibitory effects of \(\omega\)-conotoxin GVIA.

There are many similarities between synaptic transmission from splanchnic nerves to chromaffin cells and sympathetic ganglionic transmission (17). In isolated guinea pig paravertebral ganglia, an electrophysiological study has shown that both N- and P-type Ca\(^{2+}\) channel antagonists reduce cholinergic synaptic conductance, whereas L-type Ca\(^{2+}\) channel antagonist does not (19). In isolated rat superior cervical ganglia, both N- and P-type Ca\(^{2+}\) channel antagonists inhibit the rise in Ca\(^{2+}\) concentration in the terminal boutons (22). Moreover, in isolated rat superior cervical ganglia, \(^{3}\text{H}\)-labeled ACh release induced by high K\(^{+}\) is inhibited by both N- and P-type Ca\(^{2+}\) channel antagonists but unaffected by L-type Ca\(^{2+}\) channel antagonist (15). Our findings are similar to these findings obtained from isolated sympathetic preganglionic nerves.

The inhibition by \(\omega\)-conotoxin GVIA (50 \(\mu\)g/kg) was almost the same as that by \(\omega\)-conotoxin GVIA (10 \(\mu\)g/kg). Moreover, the inhibition by combined \(\omega\)-conotoxin GVIA (50 \(\mu\)g/kg) and MVIIIC (250 \(\mu\)g/kg) was almost algebraically the sum of the individual inhibition by \(\omega\)-conotoxin GVIA (10 \(\mu\)g/kg) and MVIIIC (50 \(\mu\)g/kg). These results suggest that fivefold higher doses of \(\omega\)-conotoxin GVIA and MVIIIC are sufficient to cause inhibition of Ca\(^{2+}\) channels. However, \(\sim 30\%\) of ACh release was resistant to combined \(\omega\)-conotoxin GVIA (50 \(\mu\)g/kg) and MVIIIC (250 \(\mu\)g/kg). Other types of Ca\(^{2+}\) channels except for P/Q-types may be involved in ACh release from splanchnic nerve endings. Further examination could be needed.

**Effects of Ca\(^{2+}\) channel antagonists on catecholamine release from chromaffin cells.** In the present study, nifedipine (300 \(\mu\)g/kg) did not change ACh release at 2 and 4 Hz but inhibited Epi release at 4 Hz by \(\sim 20\%\). A threefold higher dose of nifedipine (900 \(\mu\)g/kg) did not change ACh release at 2 and 4 Hz but inhibited NE and Epi releases at 4 Hz by \(\sim 30\%\). Adrenal chromaffin cells are divided into two populations: NE- and Epi-storing cells (10). L-type Ca\(^{2+}\) channels could be present on the surface of both NE- and Epi-storing cells and play a role in NE and Epi releases.

Approximately 70% of catecholamine release was resistant to nifedipine (900 \(\mu\)g/kg). This result suggests that other types of Ca\(^{2+}\) channels except for L-type are present on chromaffin cells and involved in NE and Epi releases, although we cannot exclude the possibility of incomplete inhibition of L-type Ca\(^{2+}\) channels. Species differences in the types of Ca\(^{2+}\) channels controlling Ca\(^{2+}\) influx and catecholamine release have been
shown with rat, cat, and bovine chromaffin cells (5, 6, 13, 24).
In patch-clamp studies of isolated rat chromaffin cells, Ca\(^{2+}\) inward current elicited by depolarization is sensitive to both L- and N-type Ca\(^{2+}\) channel antagonists (16, 21). The study measuring Ba\(^{2+}\) current by patch-clamp technique has shown the existence of L-, N-, and P/Q-type Ca\(^{2+}\) channels on rat chromaffin cells and the following distribution of Ca\(^{2+}\) channels in decreasing order: L-type > N-type > P/Q-type (13). In the present study, \(\omega\)-conotoxin GVIA (10 and 50 \(\mu g/kg\)) inhibited NE release at 4 Hz and Epi release at 2 and 4 Hz by approximately 45–50%. \(\omega\)-Conotoxin MVIIIC (50 \(\mu g/kg\)) inhibited Epi release at 4 Hz by \(\sim\)30%. Combined \(\omega\)-conotoxin GVIA (50 \(\mu g/kg\)) and MVIIIC (250 \(\mu g/kg\)) inhibited NE and Epi releases at 2 and 4 Hz by approximately 75–85%. However, these Ca\(^{2+}\) channel antagonists simultaneously inhibited ACh release to almost the same extent. It is difficult to determine how much Ca\(^{2+}\) antagonists are acting on chromaffin cells when Ca\(^{2+}\) channel antagonists inhibit ACh release. Thus, although much of these inhibitions of catecholamine release may be considered to be consequences of the inhibition of ACh release, we cannot exclude the possibility that N- or P/Q-type Ca\(^{2+}\) channels may be involved in the in vivo catecholamine release on chromaffin cells.

The inhibition of NE release at 2 Hz by \(\omega\)-conotoxin GVIA (10 and 50 \(\mu g/kg\)) and the inhibition of NE release at 4 Hz by \(\omega\)-conotoxin MVIIIC (50 \(\mu g/kg\)) were not statistically significant despite significant inhibitions of ACh and Epi releases. In the same preparation, we have shown that cholinergic antagonists almost inhibited NE and Epi releases induced by nerve stimulation (1, 2). However, the correlation between ACh and NE releases was poorer than that between ACh and Epi releases when stimulation frequency was raised stepwise (2). Insignificant inhibitions of NE release may be ascribed to this poor correlation.

In the present study, Ca\(^{2+}\) channel antagonists did not affect basal dialysate NE and Epi levels. In our previous study of the same preparation, these basal levels were not affected by neostigmine, hexamethonium, or atropine (1). We then concluded that these basal dialysate NE and Epi levels reflect noncholinergic catecholine release. N-, P/Q-, and L-type Ca\(^{2+}\) channels may not play a major role in basal noncholinergic catecholine release from adrenal medulla.

**Methodological considerations.** We administered neostigmine locally to adrenal medulla through a dialysis probe. Cholinesterase inhibitor was necessary to monitor endogenous ACh even during splanchnic nerve stimulation because released ACh is rapidly degraded by acetylcholinesterase before reaching the dialysis fiber. In the same preparation, local administration of neostigmine enhanced the dialysate catecholamine response to nerve stimulation by approximately threefold, but dialysate ACh and catecholamine responses are correlated with the stimulation frequency of splanchnic nerves in the presence of neostigmine (2). Thus dialysate ACh and catecholamine responses are likely to be correlated with the amount of Ca\(^{2+}\) influx from voltage-dependent Ca\(^{2+}\) channels even in the presence of neostigmine.

Intravenous administration of Ca\(^{2+}\) channel antagonists induced changes in heart rate or mean arterial pressure. These changes might affect ACh and catecholamine releases through a baroreflex mechanism. Moreover, these hemodynamic changes might decrease the spillover of ACh or catecholamine from adrenal medulla and affect the dialysate ACh or catecholamine concentrations (20). In our preparation, however, splanchnic nerves had been transected before control sampling, and basal dialysate catecholamine concentrations did not change before or after administration. Thus effects of these hemodynamic changes could be negligible when we considered the effects of Ca\(^{2+}\) channel antagonists on nerve stimulation-induced dialysate responses.

In conclusion, we applied dialysis technique to the adrenal medulla of anesthetized rats and investigated the effects of Ca\(^{2+}\) channel antagonists on ACh and catecholamine releases induced by electrical stimulation of splanchnic nerves. Both N- and P/Q-type Ca\(^{2+}\) channels control ACh release on preganglionic splanchnic nerve endings while L-type Ca\(^{2+}\) channels do not. L-type Ca\(^{2+}\) channels are involved in norepinephrine and epinephrine releases on chromaffin cells.

**GRANTS**

This study was supported by the Program for Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research (of Japan); by a Health Sciences Research Grant for Advanced Medical Technology from the Ministry of Health and Welfare of Japan; by a Ground-Based Research Grant for the Space Utilization promoted by the National Space Development Agency of Japan and Japan Space Forum; and by grants-in-aid for scientific research from the Ministry of Education, Science.

**REFERENCES**

15. González Burgos GR, Biati FI, Cherksey BD, Sugimori M, Linas RR, and Uchitel OD. Different calcium channels mediate transmitter release


