Modulatory Action of Prostaglandin D2 on the Release of 3H-Norepinephrine from Rat Cerebellar Slices

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Abstract—The modulatory action of prostaglandin D2 (PGD2) on the high-K+-induced release of 3H-norepinephrine (3H-NE) from rat cerebellar slices was investigated in relation to the presynaptic feedback mechanisms for the NE release. PGD2 (10^{-7}–10^{-5} M) dose-dependently suppressed the release of 3H-NE. The 3H-NE release was also dose-dependently decreased by 10^{-10}–5×10^{-8} M clonidine and increased by 10^{-9}–10^{-8} M yohimbine, and there was an antagonism between clonidine and yohimbine, indicating the presence of an α2-adrenoceptor-mediated negative feedback mechanism in the rat cerebellum. The inhibitory action of clonidine was not additive to that of PGD2, while there appeared to be an additivity between the effects of PGD2 and yohimbine. The 3H-NE release was increased by I-isoproterenol and decreased by I-propranolol, but only at concentrations higher than 10^{-6} M. PGD2 nearly abolished the actions of these β-adrenergic agents, and the 3H-NE release remained at a level similar to that induced by 10^{-5} M PGD2 alone. Based on these results, it was tentatively suggested that PGD2 inhibits the 3H-NE release by a mechanism independent of adrenoceptor-mediated feedback mechanisms.

Prostaglandins (PGs) are known to be present not only in peripheral neurons but also in the central nervous system (1–5). Regarding PGD2 in particular, the enzyme system and the binding protein for this PG have been demonstrated to exist at high concentrations in mammalian brains (5, 6). As for the cerebellum, it has been reported that both PGD2 binding protein and PGD2 degrading enzymes are present in Purkinje cell somata in the miniature pig (7) and also that PGD2 potentiates the postsynaptic actions of transmitter amino acids on Purkinje cells (8, 9).

We have previously found that the high-K+-evoked release of 3H-NE from rat cerebellar slices is significantly suppressed by PGD2 (10). Similar suppressive actions of other PGs such as PGEs have also been reported in peripheral sympathetic (11–14) and central catecholamine (15–17) neurons.

The present study was carried out to confirm the suppressive effect of PGD2 on the release of 3H-NE and to examine whether control mechanisms for the NE release are equipped in the rat cerebellum and whether these mechanisms, if present, are related to the PGD2 action.

Materials and Methods

High-K+-induced release of 3H-NE from rat cerebellar slices: Male Wistar rats (about 250 g) were killed by decapitation and the cerebellum removed. The vermis portion of the cerebellum was sagittally chopped at the thickness of 300 μm using a McIlwain chopper (18). Several cerebellar slices were preincubated under gentle bubbling with 95% O2/5% CO2 gas for 15 min at 37°C in 5 ml of Krebs-Ringer bicarbonate medium (control medium) of the following composition: 125 mM NaCl, 5 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 1 mM NaH2PO4, 24 mM NaHCO3, 11 mM glucose, 1 mM ascorbic acid and 0.01 mM pargyline, saturated with 95% O2/5% CO2 gas, the pH being 7.4,
Then, \(^{3}\)H-NE (5 \(\mu\)Ci/5 \(\mu\)l, final concentration=1.81\(\times\)10\(^{-8}\) M) was added, and the incubation was continued for 30 min to load the slices with \(^{3}\)H-NE. \(^{3}\)H-NE loaded slices were rinsed thoroughly with chilled control medium, and the slice was mounted (one slice for each experiment) in the superfusion chamber (an acrylic cylinder, 12 mm i.d. \(\times\)6 mm, dead space=0.6 ml, two nylon meshes for holding the slice). To measure the spontaneous release of \(^{3}\)H-NE, control medium was superfused at a constant flow rate of 2 ml/min for 20 min, and eluates were collected every minute. To induce the evoked-release of \(^{3}\)H-NE, control medium was superfused for 12 min; then a high-K\(^{+}\) medium (5 mM KCl and 125 mM NaCl in control medium were raised to 25 mM and decreased to 105 mM, respectively) was applied for 8 min at a constant flow rate of 2 ml/min. All drugs tested were dissolved in the high-K\(^{+}\) medium. When the release of \(^{3}\)H-NE was examined in the Ca\(^{2+}\)-free condition, Ca ions in the control medium and in the high-K\(^{+}\) medium were replaced by 1 mM EGTA. Slices after superfusion were dissolved in 0.5 ml Protosol, and the radioactivity was counted. The radioactivity released into each eluate fraction was expressed as the percentage of the total radioactivity accumulated in the slice during the loading incubation (see Fig. 1). The total radioactivity released for the 8 min stimulation period was also calculated and expressed as the percentage of the total radioactivity in the slice (see Tables 1 and 2).

**High-K\(^{+}\)-induced release of \(^{3}\)H-NE from rat cerebellar P\(_{2}\) fraction:** The P\(_{2}\) fraction was prepared from isolated rat cerebellum according to the method of Gray and Whittaker (19). The P\(_{2}\) fraction suspended in control medium (about 3 mg protein/5 ml) was preincubated under gentle bubbling with 95% O\(_{2}\)/5% CO\(_{2}\) gas for 15 min at 37\(^\circ\)C; then \(^{3}\)H-NE (5 \(\mu\)Ci/5 \(\mu\)l) was added, and the incubation was continued for 30 min to load the P\(_{2}\) fraction with \(^{3}\)H-NE. The \(^{3}\)H-NE loaded P\(_{2}\) fraction was centrifuged, and then the pellet was suspended in 0.5 ml control medium and adsorbed to a glass filter (Whatman GF/B). The filter was mounted in the superfusion chamber, and superfusion was made in the same manner as for slice preparations.

\(^{3}\)H-NE (levo-[\(\alpha\)-ring-2,5,6-\(^{3}\)H]-norepinephrine, specific activity=52.9 Ci/m mole, NET-678) and Protosol were obtained from NEN (U.S.A.), and ACS II (scintillation cocktail) was from Amersham (U.S.A.). Prostaglandin D\(_{2}\) was purchased from Funakoshi Chemicals (Tokyo). Clonidine-HCl, I-isoproterenol-HCl and pargyline-HCl were obtained from Sigma Chemicals (U.S.A.). I-Propranolol-HCl was kindly donated by ICI Pharmaceutical (Tokyo). All other chemicals used were obtained from Wako Pure Chemicals Industries (Tokyo).

**Results**

**Effect of PGD\(_{2}\) on the release of \(^{3}\)H-NE**

As shown in Fig. 1A, the application of 25 mM KCl in the presence of 2 mM Ca\(^{2+}\) induced a marked increase of the release of \(^{3}\)H-NE from a rat cerebellar slice (solid line with open circles). The % total radioactivity released by 8 min high-K\(^{+}\) stimulation was 15.72\(\pm\)0.64 (\(\pm\)S.E.)% (n=32) of the total radioactivity in the slice as shown in Table 1. This K\(^{+}\)-evoked release of \(^{3}\)H-NE was dose-dependently suppressed by PGD\(_{2}\): by about 19% (to 12.69\(\pm\)1.37% (8), P<0.2, not significant) with 10\(^{-7}\) M PGD\(_{2}\) (Fig. 1A, solid line with closed squares), by about 25% (to 11.86\(\pm\)1.01% (8), P<0.01) with 10\(^{-6}\) M PGD\(_{2}\) (Fig. 1A, solid line with open squares), and by about 52% (to 7.59\(\pm\)0.27% (12), P<0.001) with 10\(^{-5}\) M PGD\(_{2}\) (Fig. 1A, solid line with closed circles) (see also Table 1). The P-values (two-tailed Student's t-test) in parentheses above and in the following text are for the difference in the values of % total radioactivity released by high-K\(^{+}\) between the test and the control (see Table 1), unless otherwise stated.

In the absence of Ca\(^{2+}\), the release of \(^{3}\)H-NE was reduced to about 1/8 of that in the presence of 2 mM Ca\(^{2+}\) (Fig. 1A, dotted line with open circles), indicating the synaptic origin of the \(^{3}\)H-NE release. PGD\(_{2}\) at 10\(^{-5}\) M did not depress this Ca\(^{2+}\)-independent release of \(^{3}\)H-NE (Fig. 1A, dotted line with closed circles).

The high-K\(^{+}\)-evoked release of \(^{3}\)H-NE from rat cerebellar P\(_{2}\) fraction is shown in
Effect of PGD$_2$ on Norepinephrine Release

Fig. 1. Effect of PGD$_2$ on high-K$^+$-evoked release of $^3$H-NE from cerebellar slices (A) and cerebellar P$_2$ fraction (B) from rats. Cerebellar slices or P$_2$ fraction loaded with $^3$H-NE were superfused (2 ml/min) with Krebs-Ringer medium for 12 min, then with isotonic 25 mM K$^+$ medium with or without PGD$_2$ for 8 min as marked by horizontal bars. The superfusate was collected every minute for 20 min. Ordinate scales: percentage radioactivity released in each fraction. The total radioactivity accumulated in the slice or P$_2$ fraction during the loading incubation period was taken as 100%. Abscissa scales: superfusion time (min). In A and B, ○: control (PGD$_2$ absent), ■: +10$^{-5}$ M PGD$_2$, □: +10$^{-6}$ M PGD$_2$, ●: +10$^{-5}$ M PGD$_2$. Dotted lines in A are in the absence of Ca$^{2+}$. The dotted and solid curves without symbols in A and B indicate spontaneous (unstimulated) releases in the absence and presence of 2 mM Ca$^{2+}$, respectively. The values plotted are the means±S.E. of 4–24 observations.

Fig. 1B. In the presence of 2 mM Ca$^{2+}$, about 17% of the radioactivity in the P$_2$ fraction used was released by 25 mM K$^+$-stimulation (Fig. 1B, open circles), and this release was significantly (P<0.001) suppressed by about 43% with 10$^{-5}$ M PGD$_2$ (Fig. 1B, closed circles).

Effects of $\alpha$-adrenergic agents on the $^3$H-NE release

In the absence of PGD$_2$: As shown in Table 1, clonidine (an $\alpha_2$-agonist) dose-dependently suppressed the high-K$^+$-evoked release of $^3$H-NE from rat cerebellar slices. The suppression became obvious at 10$^{-9}$ M of clonidine and reached a plateau level of about -50% at 10$^{-8}$–10$^{-6}$ M. On the other hand, 10$^{-10}$–10$^{-6}$ M yohimbine, an $\alpha_2$-antagonist, dose-dependently increased the $^3$H-NE release as shown in Table 1. In the presence of 5×10$^{-9}$ M clonidine, yohimbine at the concentration of 10$^{-8}$–10$^{-6}$ M did not increase the $^3$H-NE release, but moderately.
suppressed it (Table 1).

**In the presence of PGD₂**: As shown in Table 1, when 10⁻⁶ M PGD₂ was present, the suppressive action of clonidine was attenuated rather in a non-linear fashion, e.g., 10⁻⁵ M PGD₂ reduced the 10⁻⁹ M clonidine-induced % change of about −23% to about −9% and the 10⁻⁶ M clonidine-induced % change of about −50% to about −41%. It may be also said from Table 1 that the suppressive action of PGD₂ was reduced largely (from about −52% to −9%) by the presence of 10⁻⁹ M clonidine and slightly (from about −52% to −41%) by 10⁻⁶ M clonidine.

The facilitating action of yohimbine was also influenced by 10⁻⁵ M PGD₂. As shown in Table 1, the significant increases of the ³H-NE release induced by yohimbine (about 47% and 52% with 10⁻⁸ M and 10⁻⁷ M yohimbine, respectively) were abolished by 10⁻⁵ M PGD₂, and the effect of 10⁻⁶ M yohimbine was also largely reduced. In particular, the increase (about 52%) produced by 10⁻⁷ M yohimbine appeared to be neutralized by the suppression (about −52%) by 10⁻⁶ M PGD₂, probably suggesting that the actions of yohimbine and PGD₂ were additive.

**Effects of β-adrenergic agents on the ³H-NE release**

**In the absence of PGD₂**: I-Isoproterenol (a non-selective β-agonist) increased the ³H-NE release in a dose-dependent manner only at high concentrations (2×10⁻⁸–5×10⁻⁵ M) as shown in Table 2. A non-selective β-antagonist, I-propranolol, on the other hand, dose-dependently decreased the ³H-NE release, but again only at high concentrations such as 5×10⁻⁶ M and 2×10⁻⁵ M (Table 2). In the presence of 2×10⁻⁶ M I-isoproterenol and 10⁻³–2×10⁻⁵ M I-propranolol, there occurred an apparent antagonism between them as shown in Table 2.
Table 2. Effects of PGD2 and β-adrenergic agents on high-K+-evoked release of 3H-NE from rat cerebellar slices

| Adrenergic agents added (M) | % Total radioactivity released by high-K+ | In the absence of PGD2 | In the presence of 10^{-6} M PGD2 |
|---------------------------|----------------------------------------|------------------------|-------------------------------|
| None (Control)            | 15.7±0.64%(32) [0.0%]                  | 7.59±0.27%(12)* [-51.7%] < 0.0% > |
| I-Isoproterenol 10^{-6}   | 16.87±0.74(6) [7.3]                    | 8.84±0.50(4)* [-43.8%] < 16.5% > |
| 2·10^{-6}                 | 23.78±1.12(6)* [51.3]                  | 8.36±1.06(4)* [-46.8%] < 16.1% > |
| 5·10^{-6}                 | 32.26±1.01(3)* [105.2]                 | 8.50±0.57(3) ** [-45.9%] < 12.0% > |
| I-Propranolol 2·10^{-7}   | 16.10±1.58(4) [2.4]                    | 7.30±0.90(3)* [-53.6%] < 3.8% > |
| 10^{-6}                   | 11.99±1.58(4) [-23.7]                  | 8.09±2.87(4)* [-47.3%] < 6.6% > |
| 2·10^{-6}                 | 12.38±0.23(4) [-21.2]                  | 8.30±0.45(3) ** [-47.2%] < 9.4% > |
| 5·10^{-6}                 | 11.53±1.30(8) *** [-26.7]             | 6.91±0.30(4) **** [-56.0%] < 9.0% > |
| 2·10^{-6}                 | 9.21±0.07(3) * [-41.4]                |                        |
| I-Isoproterenol (2·10^{-6}) + I-Propranolol 10^{-6} | 18.93±2.02(8) [7.7]             |                        |
| 5·10^{-6}                 | 11.75±1.91(8) [-25.3]                  |                        |
| 2·10^{-6}                 | 13.05±0.81(8) [-17.0]                  |                        |

See Table 1 for experimental methods and the expression of the values shown. Significant differences (two-tailed Student's t-test): *P<0.001, **P<0.005, ***P<0.01 and ****P<0.025 from 15.72±0.64(32).

In the presence of PGD2: When 10^{-6} M PGD2 was present, the enhancing effect of I-isoproterenol on the 3H-NE release was abolished, and the release was maintained at a level as low as that induced by PGD2 alone, i.e., at about -44% to -46%, as shown in Table 2. This was also the case for the suppressive effect of I-propranolol, namely the suppressive action of I-propranolol (about -24% with 10^{-6} M I-propranolol and about -41% with 2·10^{-5} M) was completely extinguished by the presence of 10^{-6} M PGD2 to the levels of -47% to -56% (Table 2).

In agreement with this concept, the high-K+-evoked release of 3H-NE from rat cerebellar slices was significantly depressed by clonidine and enhanced by yohimbine, showing an antagonism between them (Table 1). These findings indicate that presynaptic autoreceptors for NE and a negative feedback mechanism via these presynaptic receptors are equipped also in the rat cerebellum like in other central and peripheral nervous systems.

The release of NE from rat hypothalamic slices has been reported to be enhanced by β-agonists and decreased by β-antagonists (20, 27). Similar phenomena have long been known to exist in peripheral neurons (28). Based on these observations, the existence of a positive feedback control mechanism via β-receptors has been proposed (20, 27, 29). In the case of rat cerebellar slices, a β-agonist (I-isoproterenol) significantly increased the 3H-NE release, while a β-antagonist (I-propranolol) decreased it, and there was an antagonism between them (Table 2). Although these findings appeared to suggest the possible existence of a similar positive feedback control mechanism also in the rat cerebellum,

Discussion

Negative and positive feedback controls of the NE release in the rat cerebellum

It is well-known that α-adrenergic antagonists facilitate the release of endogenous NE from rat hypothalamic (20) and cerebral cortical (21) slices and also that clonidine, an α2-agonist, suppresses the release of NE from rat brain slices (20, 22–24). Based on these findings, it is now thought that presynaptic α-receptors (α2-receptors) are present in the brain, and a negative feedback control mechanism for the synaptic release of NE is functioning in the central nervous system (14, 25) in a similar manner as in peripheral neurons (26).

In the presence of PGD2: When 10^{-6} M PGD2 was present, the enhancing effect of I-isoproterenol on the 3H-NE release was abolished, and the release was maintained at a level as low as that induced by PGD2 alone, i.e., at about -44% to -46%, as shown in Table 2. This was also the case for the suppressive effect of I-propranolol, namely the suppressive action of I-propranolol (about -24% with 10^{-6} M I-propranolol and about -41% with 2·10^{-5} M) was completely extinguished by the presence of 10^{-6} M PGD2 to the levels of -47% to -56% (Table 2).

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it is premature to derive such a conclusion, because the approximate lowest effective concentrations of I-isoproterenol and I-propranolol were above $10^{-6}$ M, which are 1–2 orders of magnitude higher than those used by Ueda et al. (20) and known to show a local anesthetic (membrane stabilizing) action (30). However, since there was an apparent antagonism between I-isoproterenol and I-propranolol (Table 2), a possibility that the effects of these \( \beta \)-adrenergic agents observed in the present study might be mediated, at least partly, by \( \beta \)-adrenergic receptors cannot be ignored entirely. In any case, it is necessary to observe the effects of \( \beta \)-adrenergic agents on the release of endogenous NE to prove or disprove the existence of the positive feedback mechanism in the rat cerebellum.

**PGD\(_2\) action and adrenergic receptors**

**Suppressive action of PGD\(_2\):** Reimann et al. (17) reported that the release of \(^3\)H-NE from rat cerebral cortical slices was unaffected by PGD\(_2\), while our present results showed the significant suppression by PGD\(_2\) of the \(^3\)H-NE release from rat cerebellar slices (Fig. 1). This discrepancy might be due to differences in the brain region and the PGD\(_2\) concentration used. At the concentration of \(10^{-6}\) M used by Reimann et al. (17), the suppressive effect of PGD\(_2\) could hardly be significant as indicated by the critical value of \(P<0.01\) observed with \(10^{-6}\) M PGD\(_2\) (see Results).

**\( \alpha \)-Receptors:** As shown in Table 1, both the suppressive effect of clonidine and the enhancing effect of yohimbine were attenuated by PGD\(_2\). The interaction between yohimbine and PGD\(_2\) can be the result of a functional antagonism between them, because there seemed to be some additiveness between their effects (Table 1). PGD\(_2\) apparently resembles clonidine with respect to its suppressive effect on the NE release and to the antagonistic action on the yohimbine action. However, PGD\(_2\) appears not to be acting simply as an \( \alpha_2 \)-agonist, because there was an interaction between PGD\(_2\) and clonidine (Table 1). Although the mechanism of this interaction between clonidine and PGD\(_2\) is unclear at present, it is at least inferable that the intrinsic negative feedback control via \( \alpha_2 \)-receptors of the release of endogenous NE can be suppressively modulated by the local release of PGD\(_2\) in a physiological situation.

**\( \beta \)-Receptors:** As mentioned above, the observed effects of I-isoproterenol and I-propranolol on the \(^3\)H-NE release could be non-specific. However, it was of interest that both the enhancing action of I-isoproterenol and the suppressive effect of I-propranolol on the \(^3\)H-NE release were abolished by PGD\(_2\). Unlike the case of \( \alpha \)-adrenergic agents, the level of NE release in the presence of PGD\(_2\) and a \( \beta \)-agonist or \( \beta \)-antagonist was not significantly different from that in the presence of PGD\(_2\) alone (Table 2). This suggests that the effects of \( \beta \)-adrenergic agents, whether they are mediated by \( \beta \)-adrenoceptors or not, could be extinguished by PGD\(_2\).

Since no direct information about the mechanism of the PGD\(_2\) action could be obtained by the present study, the involvement of the presynaptic PG receptors suggested by Starke (14) and of the presynaptic cyclic nucleotide system as proposed by Partington et al. (31) must be examined to explain the mechanism underlying the suppressive action of PGD\(_2\) itself and the interaction between PGD\(_2\) and adrenergic agents.

In conclusion, PGD\(_2\) effectively decreased the high-K\(^+\)-evoked release of \(^3\)H-NE from rat cerebellar slices, suggesting a possibility of physiological modulation by PGD\(_2\) of the synaptic release of NE in the cerebellum. Although this PGD\(_2\) action apparently resembled the suppressive action of clonidine, it appeared to be rather independent of any \( \alpha_2 \)-adrenergic-receptor-mediated feedback mechanism, because there seemed to be some additiveness between the effect of yohimbine and PGD\(_2\). Conclusions about the presence and absence of the positive feedback mechanism via \( \beta \)-adrenoceptors for NE release in the rat cerebellum and of an interaction between PGD\(_2\) and \( \beta \)-adrenoceptors must wait for further investigations.

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