Carteolol Is a Useful Tool to Prove the Tonic Functioning Nature of Presynaptic \( \beta \)-Adrenoceptors on Peripheral Noradrenergic Neurons but Not on Central Catecholaminergic Neurons

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Abstract—Effects of carteolol on norepinephrine (NE) release were studied at 2 Hz mainly in rat hypothalamic slices. Isoproterenol at 1 and 10 nM concentration-dependently facilitated NE release. Isoproterenol (10 nM)-induced facilitation was antagonized by 1 and 10 nM \( dl \)-carteolol, but not antagonized by 1 nM \( d \)-carteolol. \( dl \)-Carteolol alone at 1 nM to 10 \( \mu \)M did not inhibit NE release. In brainstem slices, 10 nM isoproterenol also facilitated NE release, and this facilitation tended to be antagonized by 1 nM \( dl \)-carteolol. Nanomolar concentrations of carteolol stereoselectively antagonized isoproterenol-induced facilitation of NE release via presynaptic \( \beta \)-adrenoceptors in rat hypothalamic slices.

There is a facilitatory regulatory mechanism of the release of the transmitter norepinephrine (NE) via presynaptic \( \beta \)-adrenoceptors on peripheral sympathetic nerve terminals (1–10). We have used carteolol, a non-selective \( \beta \)-antagonist, as a useful tool to determine if these adrenoceptors function tonically (9, 10), because carteolol has a far more potent antagonistic action against the isoproterenol-induced facilitation of the NE release than propranolol (8), and in fact, carteolol alone at \( 10^{-8} \) to \( 10^{-6} \) M stereoselectively and concentration-dependently inhibited the NE release in guinea pig pulmonary arteries (7). The tonic function of these adrenoceptors was also demonstrated using carteolol at \( 10^{-8} \) to \( 10^{-6} \) M in renal and mesenteric arteries from young spontaneously hypertensive rats (SHR) and age-matched Wistar Kyoto rats (WKY) (9). The subtype of these peripheral presynaptic adrenoceptors is \( \beta_2 \) in most types of preparations (2, 4, 5). On the other hand, we showed the coexistence of presynaptic \( \beta_1 \)- and \( \beta_2 \)-adrenoceptors on dopaminergic (11), noradrenergic (11, 12) and adrenergic (13) neuron terminals in rat hypothalamic slices. These adrenoceptors are functioning tonically, since propranolol alone at \( 3 \times 10^{-7} \) or \( 3 \times 10^{-6} \) M inhibited the evoked release of catecholamines in a stereoselective manner. Mainly in superfused hypothalamic slices of rats, we have attempted to determine if carteolol could be a better tool than propranolol for proving the tonically functioning nature of central presynaptic \( \beta \)-adrenoceptors.

Male Sprague-Dawley rats weighing 200 to 250 g were decapitated, and the hypothalamus or the brainstem was dissected, sliced and superfused in a glass chamber at a rate of 0.45 ml/min at 37°C with Krebs medium bubbled with 5% CO\(_2\) in O\(_2\) in the presence of 20 \( \mu \)M cocaine. Bipolar electrical field stimulations (2 Hz, 2 msec, 25 V, 3 min) were done twice, at 60 (S\(_1\)) and 90 (S\(_2\)) min, after the start of superfusion through platinum spiral electrodes set up at the 2 ends of the chamber, using an electrical stimulator with an isolator. Samples were collected every 3 min throughout the experiments. NE was measured using high-performance liquid chromatography with an electrochemical detector (Yanako) as described previously (11, 13). The evoked release (S) of NE was calculated by subtracting the estimated basal release from the total release in 3 successive samples during and after stimulation. The
release in a sample immediately before stimulation was regarded as the spontaneous release (Sp). Isoproterenol or carteolol alone was applied 15 min before S2, and the effect was evaluated by the release ratios S2/S1 and Sp2/Sp1. Carteolol was also pretreated before S1 and was present throughout the experiments. Drugs used were dl- and d-carteolol hydrochloride (Otsuka) and l-isoproterenol hydrochloride (Sigma), the former dissolved in distilled water and the latter with 0.1 N HCl to minimize any oxidation. Data shown are the mean±S.E., and statistical significance was calculated by Student's t-test.

In control slices of hypothalamus (n=7) and brainstem (n=7), Sp1 of NE was 0.401±0.084 pmol and 0.339±0.093 pmol. S1 was 0.889±0.169 pmol and 0.247±0.034 pmol, and the tissue content of NE after the superfusion experiments was 161.4±19.9 pmol and 43.7±7.4 pmol, respectively. Sp2 was slightly attenuated, and Sp2/Sp1 was 0.68±0.04 in hypothalamic slices and 0.75±0.08 in brainstem slices. S2 was also slightly reduced, and S2/S1 is shown in Table 1. Isoproterenol alone, carteolol alone and the combined application produced no effect on Sp2/Sp1 and the tissue content of NE. S1 and Sp1 were also not modified by the pretreatment with carteolol.

In hypothalamic slices, isoproterenol at 1 and 10 nM facilitated the release of NE in a concentration-dependent manner (Table 1). This isoproterenol (10 nM)-induced facilitation was antagonized by 0.1 and 1 nM dl-carteolol in a concentration-dependent manner. A ceiling phenomenon of this antagonism was seen with 10 nM dl-carteolol. On the other hand, 1 nM d-carteolol produced no antagonism. This stereoselective antagonism by the low concentrations of carteolol further supports the existence of presynaptic β-adrenoceptors on noradrenergic neuron terminals in rat hypothalamus (11, 12) and agrees with our previous findings in guinea pig pulmonary arteries (8). In brainstem slices, isoproterenol at 3 and 10 nM facilitated the NE release in a concentration-dependent manner, and this is consistent with the findings in the same preparations from young and adult WKY (14). This facilitation tended to be antagonized by dl-carteolol at 1 nM.

In hypothalamic slices (Table 2), however, a wide concentration range of dl-carteolol alone produced no inhibition of the NE release. This is not consistent with the tonically functioning nature of presynaptic β-adrenoceptors on central catecholaminergic

### Table 1. Stereoselective antagonism by carteolol against isoproterenol-induced facilitation of the evoked release of norepinephrine (NE) from slices of rat hypothalamus and brainstem

| Regions   | Pretreatments (nM) | Isoproterenol (nM) | n | Release ratio (S2/S1) |
|-----------|-------------------|-------------------|---|----------------------|
| Hypothalamus | None              | Control           | 5 | 0.89±0.04            |
|           |                   | 1                 | 4 | 1.08±0.11            |
|           |                   | 10                | 9 | 1.16±0.05**          |
|           | d/-Carteolol 0.1  | 10                | 4 | 1.08±0.13            |
|           |                   | 1                 | 6 | 0.77±0.06††††        |
| Brainstem  | None              | Control           | 7 | 0.88±0.05            |
|           |                   | 3                 | 5 | 0.97±0.07            |
|           |                   | 10                | 8 | 1.11±0.08*           |
|           | d/-Carteolol 1    | 10                | 7 | 0.94±0.05            |

Biphasic electrical field stimulation (2 Hz, 2 msec, 25 V, 3 min) was done twice in the presence of 10 μM cocaine at 60 (S1) and 90 (S2) min after the start of superfusion. Isoproterenol was applied 15 min before S2. Carteolol was applied before S1 and was present throughout the experiments. Data shown are the means±S.E. of the ratio of NE released by the S2 and S1 stimulations (S2/S1), and n is the number of estimations. *P<0.05, **P<0.01, compared to the control; †P<0.05, ††P<0.01, compared to 10 nM isoproterenol alone.
Table 2. Effects of dl-carteolol alone on the evoked release of NE from rat hypothalamic slices

| dl-Carteolol (nM) | n  | Release ratio (S2/S1) |
|------------------|----|----------------------|
| None             | 7  | 0.96±0.05            |
| 1                | 6  | 0.92±0.12            |
| 10               | 6  | 0.96±0.08            |
| 100              | 4  | 0.89±0.11            |
| 1000             | 4  | 1.05±0.26            |
| 10000            | 4  | 0.85±0.24            |

dl-Carteolol was applied 15 min before the S2 period of field stimulation. Other details are as in Table 1.

neurons, which had been indicated by the use of propranolol in the same preparations (11–13), or on peripheral noradrenergic neurons, indicated by carteolol itself in guinea pig pulmonary arteries (7, 10), renal and mesenteric arteries from young SHR and WKY (9). Key points to resolve this discrepancy appear to be firstly the coexistence of central presynaptic β1- and β2-adrenoceptors (11–13). Furthermore, the peripheral presynaptic β-adrenoceptors are mainly the β2-subtype (2, 4, 5), while the sympathomimetic action of carteolol shows β1-subtype characteristics (15). It seems likely that probable carteolol-induced tonic inhibition of the NE release may be balanced by facilitation of the release via activation of these β1-adrenoceptors by this antagonist itself in hypothalamic slices. This idea is consistent with our findings (6, 9) that although isoproterenol facilitated the NE release (6), carteolol alone at 10^-8 to 10^-6 M produced no inhibition of the NE release in spleen strips from young SHR and WKY (Y. Misu et al., unpublished observations), in which we demonstrated the existence of at least presynaptic β1-adrenoceptors, exceptionentially in the peripheral sympathetic nervous system (2).

In conclusion, nanomolar concentrations of carteolol stereoselectively antagonized the isoproterenol-induced facilitation of the NE release via presynaptic β-adrenoceptors in rat hypothalamic slices. The use of carteolol provided no evidence for the tonic function of these adrenoceptors.

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