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Short Communications

Secretion from adrenaline- and noradrenaline-storing adrenomedullary cells is regulated by a common dihydropyridine-sensitive calcium channel

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Dihydropiridines (+)-PN200-110 and (±)-Bay-K-8644 inhibit or potentiate, respectively, catecholamine release evoked by DMPP- or K-stimulation of perfused cat adrenal glands. Since both, secretion of adrenaline and noradrenaline are equally affected, and these two drugs specifically act on voltage-dependent chromaffin Ca channels, it seems that secretion of each amine from their respective cell is regulated by the same type of channel.

Separate adrenomedullary chromaffin cells containing noradrenaline or adrenaline were first visualized by Hillarp and Hökfelt[14]. Selective release of one or the other amine in response to various stimuli has been repeatedly demonstrated in the cat[1–4,6–8,16]. This might have physiological significance in the light of the fact that target adrenoceptors for both catecholamines have different tissue distribution and that noradrenaline and adrenaline preferentially stimulate α or β adrenoceptors. Since acetylcholine and high K[5] as well as many other secretagogues[13] cause secretion by Ca-dependent mechanisms, it is obvious that different voltage-dependent Ca channels on the plasma membrane of noradrenaline- or adrenaline-containing cells might be responsible for the differential secretion of catecholamines. If so, the selective secretion of adrenaline and noradrenaline should be affected differently by Ca-channel modulatory drugs. In this communication we demonstrate that in the perfused cat adrenal gland, the dihydropyridines (DHP) Ca channel activator, (±)-Bay-K-8644, and the blocker (+)-PN200-110 equally enhance or inhibit, respectively, dimethylphenylpiperazinium (DMPP) or high K-evoked adrenaline and noradrenaline release.

Cat adrenal glands were isolated[11] and perfused at room temperature (22 ± 2 °C) and 1 ml/min with Krebs-Tris solution of the following composition (mM): NaCl 134, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, glucose 11, and Tris 10. The solution was bubbled with pure O_2 and the pH adjusted to 7.4 with HCl. High K solutions contained 35.5 mM KCl with iso-osmotic reduction of NaCl. DMPP iodide was directly dissolved in Krebs solution; (+)-PN200-110 and (±)-Bay-K-8644 were dissolved in ethanol (10^{-2} M) and diluted in Krebs. Both, the solutions and the experiments were made under sodium light.

The experimental protocol consisted of an initial perfusion equilibration period (1 h) followed by stimulation pulses of DMPP (5 μM, 1 min) or K (35.5

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TABLE I

Adrenaline and noradrenaline release in basal, K- or DMPP-stimulated cat adrenal medullae perfused with Krebs solution

K (35.5 mM) or DMPP (5 μM) were applied during a 60-s period. Data are means ± S.E.M. of the number of experiments shown in parentheses.

	Noradrenaline ($ng \cdot 4\ min^{-1}$)	*Adrenaline* ($ng \cdot 4\ min^{-1}$)	*% Total*	
			Noradrenaline	*Adrenaline*
Basal	34 ± 7 ($n = 21$)	43 ± 7 ($n = 21$)	44	56
K	1831 ± 164 ($n = 12$)	1330 ± 100 ($n = 12$)	58	42
DMPP	1543 ± 214 ($n = 13$)	1649 ± 229 ($n = 13$)	48	52

mM, 1 min) applied at 30 min intervals to test the secretory response. Two perfusion samples were collected during each test in cold-acidified tubes (0.05 N perchloric acid, final): a 4 min sample in Krebs before the pulse (basal release) and a 4 min sample consisting of 1 min stimulation of DMPP or high-K solutions plus an additional 3 min in Krebs. Noradrenaline and adrenaline were separated by high-performance liquid chromatography and measured with an electrochemical detector[2]. Each gland was stimulated 5 times with DMPP or K, either in the absence or the presence of cumulative concentrations of (+)-PN200-110 or (±)-Bay-K-8644. Data are means ± S.E.M. of the individual amine release obtained in each stimulation period (S_1–S_5) after subtracting the basal release. These data are plotted as percentage of the catecholamine release in S_1. The concentration–response curve, in presence of DHP, represents adrenaline or noradrenaline release in each individual pulse expressed as percentage of the same stimulation period in control glands (in the absence of dihydropyridine).

Upon stimulation with high-K (35.5 mM for 1 min), noradrenaline release rose from 34 to 1.831 ng, an amount equivalent to 58% of total catecholamines released; the remaining 42% (1.330 ng) accounted for adrenaline (Table I). When this pulse was repeated at 30 min intervals, the size of the secretory response gradually decreased to reach 50% of S_1 at the fifth stimulus (Fig. 1A); both catecholamines decayed in a parallel manner. PN200-110 inhibited, and Bay-K-8644 enhanced K-evoked secretion in a concentration-dependent manner. Modifications in adrenaline and noradrenaline secretory responses were very close for both catecholamines (Fig. 1B).

DMPP (5 μM for 1 min) also enhanced catecholamine release in a comparable manner to high-K stimulation. In 13 experiments, noradrenaline released was 1.543 ng and adrenaline secreted amounted to 1.649 ng (Table I). When such a stimulation pattern was repeated at 30 min intervals, secretion gradually decreased to reach around 45% of S_1 at the fifth stimulus; both, adrenaline and noradrenaline release desensitized in a parallel manner (Fig. 2A). (+)-PN200-110 inhibited and (±)-Bay-K-8644 enhanced DMPP-evoked secretion in a concentration-depen-

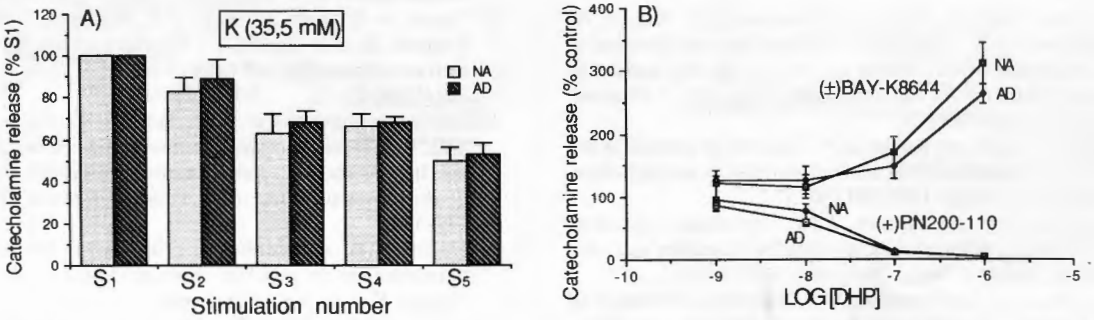

Fig. 1. A: adrenaline (AD) and noradrenaline (NA) release induced by 5 stimulation periods with K^+ (35.5 mM, 1 min) applied at 30 min intervals (S_1–S_5). Data are means ± S.E.M. of 5 experiments and are plotted as percentage of amine release during S_1. B: effects of increasing concentrations of PN200-110 and Bay-K-8644 on AD and NA release evoked by five K pulses (35.5 mM, 1 min). Data are means ± S.E. of 4 experiments and represent the effect of DHP on AD or NA release in each individual pulse expressed as percentage of the same stimulation period in control glands (absence of DHP).

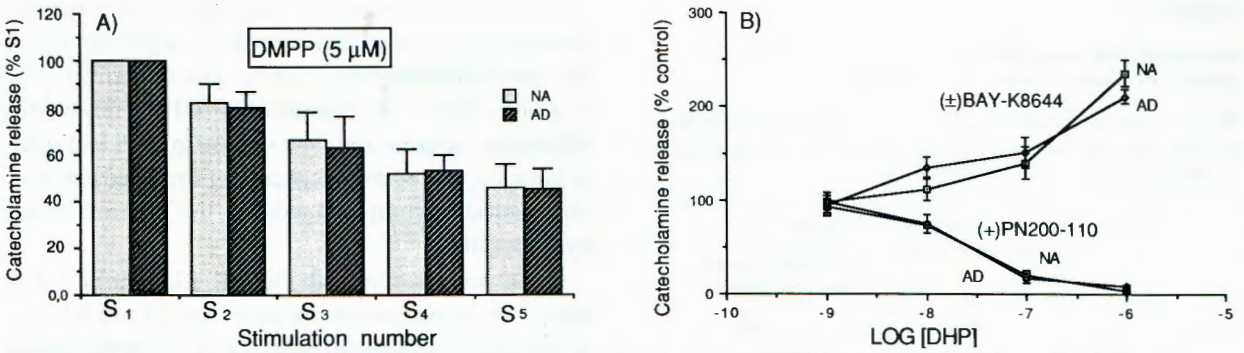

Fig. 2. A: adrenaline (AD) and noradrenaline (NA) release evoked by 5 pulses of DMPP (5 μM, 1 min) applied at 30 min intervals (S_1–S_5). Data are means ± S.E.M. of 5 experiments and are plotted as percentage of catecholamine release during S_1. B: effects of increasing concentrations of PN200-110 and Bay-K-8644 on AD and NA release evoked by 5 stimulations with DMPP (5 μM, 1 min). Data are means ± S.E.M. of 4 experiments and represent the effect of DHP on AD or NA release in each individual pulse expressed as percentage of the same stimulation periods in control glands (in the absence of DHP).

dent manner. Such modifications of secretory responses were very similar for both, noradrenaline and adrenaline (Fig. 2B).

In conclusion, since Bay-K-8644 and PN200-110 specifically act on chromaffin cell Ca channels enhancing or blocking, respectively, Ca uptake and catecholamine release[9,10,12,13,15], it seems that secretion of noradrenaline and adrenaline from their respective cells is regulated by the same type of voltage- and DHP-sensitive Ca channel.

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