DOBUTAMINE-INDUCED FACILITATION OF NOREPINEPHRINE EFFLUX FROM SPIRAL STRIPS OF GUINEA PIG PULMONARY ARTERIES

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It has been reported that dobutamine acts primarily on $\beta_1$-adrenoceptors, whereas $\beta_2$- and $\alpha$-adrenoceptors are only stimulated to a slight degree; and this dopamine derivative has advantages over other catecholamines for the improvement of myocardial function in heart failure (1-4). Unlike dopamine, it does not appear to indirectly stimulate the heart by release of norepinephrine (1). In the course of studies to characterize pre-synaptic $\beta$-adrenoceptors in guinea pig pulmonary arteries (5, 6), however, we have found that moderate doses of dobutamine, used as a relatively selective $\beta_1$-agonist, markedly increased resting and impulse-evoked efflux of tritium from spiral strips preloaded with $^3$H-norepinephrine (Table 1). Furthermore, we attempted to elucidate the mechanism of dobutamine-induced increases in the resting efflux of norepinephrine in relation to the actions of cocaine and extracellular calcium.

Spirally cut strips of guinea pig pulmonary arteries were prepared as described elsewhere (5) and incubated at 37°C for 60 min with oxygenated Krebs bicarbonate solution containing $10^{-7}$ M/[7,8-$^3$H]-norepinephrine (5 $\mu$Ci/ml), 100 mg/l ascorbic acid and 1.5 mg/l EDTA. After rinsing for 10 min with norepinephrine-free solution, the strips were mounted vertically between a pair of platinum stimulating electrodes and superfused with

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**Table 1.** Effects of dobutamine on the resting and the impulse-evoked tritium effluxes in spiral strips of guinea pig pulmonary arteries preloaded with $^3$H-norepinephrine

| Dobutamine (M) | No. of estimation | Resting efflux (%) | Impulse-evoked efflux (%) |
|----------------|-------------------|--------------------|---------------------------|
| Control        | 6                 | 86.1±3.1           | 93.4±1.5                  |
| $3\times10^{-8}$ | 4                 | 102.1±19.4         | 90.2±5.0                  |
| $10^{-7}$      | 4                 | 70.9±7.0           | 109.2±6.7                 |
| $3\times10^{-7}$ | 4                 | 94.6±5.0           | 113.5±5.0*                |
| $10^{-6}$      | 4                 | 163.4±8.3*         | 132.4±5.2*                |
| $3\times10^{-6}$ | 4                 | 231.9±25.6*        | 171.0±8.6*                |
| $10^{-5}$      | 6                 | 459.8±43.8*        | 249.9±27.8*               |

Strips were exposed to 5 $\mu$Ci/ml $^3$H-norepinephrine solution for 1 hr, rinsed, set up and then superfused with Krebs solution. Dobutamine was superfused 90 min after setting up. Transmural field stimulation (5 Hz, 20 sec period) was performed twice before ($S_1$) and 30 min after ($S_2$) onset of dobutamine superfusion. Amount of resting efflux before $S_2$ and impulse-evoked efflux during $S_2$ is expressed as % of that before and during $S_1$, respectively. Data shown are the means±s.e.m. Statistical significance: *P<0.01, compared with controls.
Krebs solution at a rate of 1 ml/min using a microtube pump (MP-3, Tokyo Rikakikai). Krebs solution was maintained at 37°C, pH 7.2 to 7.4, and was bubbled with 95% O₂ and 5% CO₂. The solution had the following composition as expressed in mM: NaCl 118.4; KCl 4.7; CaCl₂ 2.5; MgCl₂ 1.18; NaHCO₃ 25; KH₂PO₄ 1.2 and glucose 11.1. The resting tension was adjusted to 0.5 g. A 90 min equilibration period was allowed before superfusion with dobutamine solution. Transmural field stimulation was performed with rectangular pulses (5 Hz, 10 V, 2 msec pulse width, 20 sec period and 30 min interval) generated by an electrical stimulator (SEN-3201, Nihon Kohden) and always begun at the onset of a sample collection period. Superfusate was collected for 1 min into a tube, 6 ml of ACS-II solution (Amersham) was added, and total ³H activities expressed as dpm were determined using a liquid scintillation spectrometer (Packard 2660). Impulse-evoked fractional increases in ³H efflux were calculated as the difference between the basal resting efflux before stimulation and the efflux during the stimulation period. Contractile responses to transmural field stimulation were recorded isotonically on an ink-writing oscillograph (Biophysiograph 180 Systems, San-ei). Calcium free medium was prepared by elimination of CaCl₂ and by addition of 0.5 mM EGTA. Experimental protocols are explained in the legends of Table 1 and Fig. 1. Drugs used were I-[7,8-³H]-norepinephrine (The Radiochemical Center, Amersham, UK), dobutamine hydrochloride (Shionogi) and cocaine hydrochloride (Takeda). The Student’s t-test was used to evaluate data. Spontaneous total tritium efflux from the spiral strips rapidly and exponentially decreased after superfusion (5). The amount

Fig. 1. Effects of calcium deprivation (□) and 10⁻⁶ (●) and 10⁻⁴ (▲) M cocaine on increases in resting tritium efflux induced by 3×10⁻⁷ M dobutamine alone (○) in spiral strips of guinea pig pulmonary arteries. Strips were pretreated with calcium free medium containing 0.5 mM EGTA or with cocaine 10 min before dobutamine superfusion. The abscissa shows time after dobutamine superfusion. Superfusate was collected before and at every 10 min after dobutamine superfusion. On the ordinate, the resting tritium efflux before dobutamine superfusion is taken as 100%. Vertical bars show the standard errors and parentheses show the numbers of estimations. Statistical significance: *P<0.05, **P<0.01, compared with dobutamine alone. Other details are as in the legend of Table 1.
of resting efflux and efflux evoked by 100 pulses was 207.1±18.1 (mean±S.E.) and 855.7±59.8 dpm/mg wet tissue (n=26), respectively, 90 min after the onset of superfusion. It has been predicted that dobutamine does not markedly increase the resting efflux or the impulse-evoked efflux, since this agent has almost no indirect action (1), and the presynaptic β-adrenoceptors in this arterial preparation were characterized mainly as a β2-type (6). However, as shown in Table 1, superfusion with dobutamine for 30 min increased both the resting efflux and the impulse-evoked efflux in a dose-dependent manner. A significant difference was seen even at a low dose of 3×10−7 M. The result that 10−7 M does not facilitate impulse-evoked efflux is consistent with what was observed in the rat portal vein (7).

In Fig. 1, the dose of 3×10−6 M dobutamine was selected because it increases contractile responses of isolated cat papillary muscles to a similar degree as those seen with the same dose of isoproterenol (1). Dobutamine-induced facilitation of resting efflux was not affected by deprivation of extracellular calcium 10 min prior to dobutamine superfusion, suggesting that the facilitation occurs independently on a calcium influx process. Furthermore, pretreatment with cocaine dose-dependently inhibited the dobutamine-induced facilitation. The inhibitory action of 10−4 M cocaine was not restored by further superfusion with dobutamine alone. The resting efflux was not modified by calcium deprivation or by cocaine treatment. It has been established that tyramine-induced increases in norepinephrine output from adrenergic nerve endings do not depend on external calcium concentrations (8) and can be prevented by cocaine (9). The present findings demonstrate that dobutamine at moderate doses has a marked indirect sympathomimetic action in this arterial preparation. This conclusion is not consistent with findings that the pretreatment of dogs with desmethylinipramine does not inhibit positive chronotropic and inotropic responses of the heart to dobutamine (1). The increases in impulse-evoked efflux suggest that dobutamine, at least, has a cocaine-like inhibitory action on re-uptake of the transmitter into adrenergic nerve endings other than the tyramine-like displacement action with norepinephrine, since the neuronal uptake mechanism of norepinephrine is inhibited by a wide range of sympathomimetic amines structurally related to norepinephrine (10). Detailed studies concerning this subject are now in progress.

In conclusion, dobutamine exerts an indirect sympathomimetic action in guinea pig pulmonary arteries.

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