Interaction of cocaine and bunaphtide on the ^{3}H-norepinephrine uptake mechanism on isolated left atrium of guinea-pig

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Summary. Bunaphtide induces a blockade in the ^{3}H-norepinephrine uptake and retention in the isolated guinea-pig left atrium. However, this blockade does not seems to be related with the cardiodepressant effect of the drug.

N(2-Diethylamine-ethyl)N(n-butyl)-α-naphtamide or bunaphtide is a new antiarrhythmic compound with properties intermediate between those of quinidine and lidocaine[1], which has been classified as a membrane stabilizer[2]. In isolated rat mesenteric arteries it has been suggested[2] that bunaphtide could reduce the uptake of norepinephrine. Similar evidence was obtained in the anesthetized rat and guinea-pig[3]. The present study was undertaken to study the effects of bunaphide on the ^{3}H-norepinephrine (^{3}H-NE) uptake and retention by the isolated left atrium of the guinea-pig.

Methods. Guinea-pig left atrium were isolated and mounted as previously described by Garcia de Jalón et al.[4] for isolated rat ventricles. In each experiment half of the atrium served as control and the other half as experimental preparation. The halves were suspended in chambers containing 10 ml of Krebs-bicarbonate solution[5] (pH 7.4) containing 10^{-5} g/ml of ethylene diamine tetraacetic acid (EDTA). The bath fluid was maintained at 37 °C and bubbled with 95% O_2/5% CO_2. Contractions were isometrically measured with a force-displacement transducer Grass FT03 and recorded on a Grass polygraph. Each half was subjected to a resting tension of 1 g and electrically driven at a rate of 30 beats/min. Under their respective conditions, the halves were incubated with 11.3 ng/ml of d,l-^{3}H-NE for 5 min and then thoroughly washed. 4 additional washes were given over the subsequent 40-min period. The catecholamines extraction was performed according to the method of Anton and Sayre[6], and radioactivity was counted in a model Mark II Nuclear Chicago L.S. Spectrometer. 1 ml of CH_3OH and 10 ml of a scintillation mixture (PPO, 4 g; dimethyl-POPOP, 0.17 g; toluene:Triton-X-100 (2:1) 1 l) were added to the eluate. An automatic external standard was used to correct the quenching. Recovery of added ^{3}H-NE was 65%. Under these conditions, the radioactivity presented in the alumina eluated cannot be ascribed to metabolites of ^{3}H-NE but to ^{3}H-NE itself[7]. ^{3}H-NE is expressed in terms of ng/g of tissue. When we refer to ^{3}H-NE uptake, we mean ^{3}H-NE uptake and retention by isolated guinea-pig atrium. The drugs used were: d,l-($^{7-3}$H) norepinephrine hydrochloride (sp. act. 11 Ci/mM; Radiochemical Centre Ltd, Amersham), cocaine hydrochloride, bunaphtide hydrochloride, reserpine, tyramine hydrochloride and propranolol hydrochloride. Concentrations are expressed in terms of the respective salts. Statistical significance was determined by Student's t-test for paired data.

Results. In 6 paired experiments, both halves were kept under standard conditions. After a 30-min period, the experimental half was treated with cocaine (10^{-4} M, for 5 min). The other half was used as control. Both halves were treated with ^{3}H-NE. The table shows a significant decrease ($p < 0.001$) in the ^{3}H-NE uptake between cocaine-treated preparations and their controls. In another 2 series of 6 experiments, the experimental halves were treated with bunaphtide (10^{-4} M and 2.5×10^{-4} M, for 5 min), instead of cocaine and the ^{3}H-NE uptake was also decreased ($p < 0.001$) with respect to their controls (table).

Bunaphtide, 10^{-4} M, induced a positive inotropic response and potentiated the response to exogenous ^{3}H-NE. The inotropic response of bunaphtide was also obtained in reserpinized animals (10 mg/kg i.p., 24 h before; tyramine, 10^{-6} M, was added to test the absence of inotropic effects) and following treatment with propranolol (10^{-6} M to 5×10^{-5} M). However, after bunaphtide 2.5×10^{-4} M, the initial positive inotropic response was rapidly masked by a negative inotropic response and the inotropic effect of ^{3}H-NE was absent.

Discussion. The results presented in this paper indicate that the antiarrhythmic drug bunaphtide is a strong blocker of the ^{3}H-NE uptake in the isolated guinea-pig atrium. The blockade of the uptake explains the cocaine-like effects observed when ^{3}H-NE was added after bunaphtide 10^{-4} M. Its positive inotropic effects does not seem to be mediated by catecholamines, since it persisted in reserpinized animals and in propranolol-treated preparations. Higher concentrations of bunaphtide (2.5×10^{-4} M) produced a negative inotropic effect, and even when the uptake inhibition increased, the inotropic response to exogenous ^{3}H-NE was absent. This suggests that the direct cardiodepressant effect induced by bunaphtide could antagonize the positive inotropic effect of increasing concentrations of ^{3}H-NE at the receptor atmosphere due to uptake inhibition. In fact, bunaphtide was 2.5 times more potent than quinidine on the inotropic effects of the isolated guinea-pig atrium[3].

Number		^{3}H-NE present during incubation	^{3}H-NE in tissue 40 min after washout (ng/g)	Inhibition (%)
6	Control	5.5×10^{-8} M	8.31 ± 1.08	-
6	Cocaine (10^{-4} M)	5.5×10^{-8} M	0.64 ± 0.07	91.9 ± 0.4
6	Control	5.5×10^{-8} M	8.98 ± 0.75	-
6	Bunaphtide (10^{-4} M)	5.5×10^{-8} M	3.62 ± 0.25	58.5 ± 3.9
6	Control	5.5×10^{-8} M	8.60 ± 1.17	-
6	Bunaphtide (2.5×10^{-4} M)	5.5×10^{-8} M	1.92 ± 0.21	76.8 ± 2.2

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