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The effect of emetine on myocardial catecholamine metabolism

D. J. MILETICH, A. D. IVANKOVIC, R. F. ALBRECHT AND E. T. TOYOOKA

Department of Anesthesiology, Michael Reese Hospital, Chicago, Illinois 60616, U.S.A.

The effects of emetine on the uptake and release of noradrenaline from the isolated, perfused, rat heart and on the *in vivo* myocardial retention of noradrenaline have been studied. Hearts were removed from animals under light ether anaesthesia, transferred to a modified Langendorff perfusing apparatus and perfused with Krebs-Ringer bicarbonate solution at a rate of 5 ml min^{-1}. The effect of emetine on the uptake of noradrenaline was determined by perfusing hearts for a period of 6 min with perfusate containing various concentrations of emetine and 100 ng ml^{-1} of [^{14}C]noradrenaline (^{14}C-NA). After 6 min, the emetine-treated hearts contained less (±)-^{14}C-NA than control hearts. The degree of reduction increased with increasing concentrations of emetine. The release of noradrenaline was determined after first perfusing hearts with ^{14}C-NA followed by perfusion with solution containing emetine (0·01 mg ml^{-1}) but free of labelled noradrenaline for periods of 10, 20 and 60 min. It was found that by 60 min emetine significantly decreased myocardial ^{14}C-NA. In the intact animal myocardial concentrations of ^{14}C-NA were reduced 90 min after intravenous ^{14}C-NA, followed immediately by intraperitoneal emetine (1 mg kg^{-1}). These results suggest that the cardiac arrhythmia and irritability seen after emetine treatment in man may be partly due to alterations in catecholamine metabolism.

Emetine toxicity, characterized by ecg abnormalities, hypotension, tachycardia, precordial pain and skeletal muscle weakness, has severely limited its usefulness in the treatment of amoebiasis. The use of dehydroemetine, while apparently better tolerated, produces similar undesirable cardiovascular side effects (Lister, 1968; Durotoye & Salako, 1971).

Pharmacologically, emetine has been shown to produce both adrenergic and cholinergic blockade (Ng, 1966), but the direct effects of emetine on myocardial noradrenaline metabolism have not been reported. We have examined the effect of emetine on the uptake and release of noradrenaline in both the isolated, perfused heart of the rat and the intact animal.

MATERIALS AND METHODS

The hearts were quickly removed from male, Sprague-Dawley rats, 200–250 g, under light ether anaesthesia and washed in cold saline solution. The hearts were perfused as described by Iversen (1963) except that a pressure transducer and a peristaltic bilateral roller pump were introduced into the perfusion line. Each heart was perfused at a constant rate of 5 ml min^{-1} at a perfusion pressure of approximately 40 mm Hg. The perfusate media was Krebs-Ringer with bicarbonate containing in addition (per litre) glucose 1 g, ascorbic acid 20 mg, and ethylenediaminetetra-acetic acid disodium (EDTA) 10 mg. The perfusates were gassed with 5% carbon dioxide in

oxygen. In each experiment the control and test hearts were first perfused for 5 to 10 min with the modified Krebs-Ringer solution to wash away blood and to allow normal rhythmic beating to commence.

The control values for the uptake of (±)-[^{14}C]noradrenaline (^{14}C-NA) (Amersham/Seale, specific activity: 56 μCi m mol^{-1}) were determined by perfusing the hearts with Krebs-Ringer solution containing 100 ng ml^{-1} of ^{14}C-NA for 6 min. The hearts were then perfused for 90 s with noradrenaline-free perfusate to remove extracellular ^{14}C-NA. After perfusion the hearts were homogenized, centrifuged at 500 g, and 0·2 ml aliquots of supernatant were pipetted into vials containing 10 ml of phosphor-ethanol liquid scintillation counting medium. The ^{14}C content was determined using a liquid scintillation spectrometer.

The 10, 20 and 60 min release of ^{14}C-NA from control and emetine-treated hearts was determined by first perfusing each heart with Krebs-Ringer solution containing 50 ng ml^{-1} ^{14}C-NA for the 10 and 20 min values and 200 ng ml^{-1} for the 60 min value for 5 min after which the hearts were perfused with Krebs-Ringer with or without emetine for 10, 20 or 60 min. At the end of each experimental period the hearts were homogenized and 0·2 ml aliquots of the supernatant were analysed for ^{14}C content as described.

The effects of emetine and guanethidine (guanethidine sulphate, CIBA) *in vivo* were evaluated using male Sprague-Dawley rats. These were weighed, anaesthetized with ether and injected with 0·3 μg ^{14}C-NA via the tail vein. Immediately after the injection of ^{14}C-NA, emetine (1 mg kg^{-1}) was injected intraperitoneally. The animals were allowed to recover and then after 90 min they were again anaesthetized with ether, killed and the hearts removed, homogenized and analysed for ^{14}C content. The same procedure was followed and a second group of animals was treated with guanethidine, the dosage of which was adjusted so as to be on an equal dose-molar basis with emetine (e.g. 0·416 mg kg) using the molecular weights of the base and alkaloid respectively not their salts. A third group of animals was injected intraperitoneally with saline solution in amounts approximating to the volumes used in the experimental groups.

Aortic pressures were continuously monitored with a Statham pressure transducer. Individual heart rates were determined from the pressure recordings. Effluent volumes were collected and measured at the end of each experiment to verify that the perfusion rate remained constant.

RESULTS

The hearts were perfused at a rate of 5 ml min^{-1} for 6 min with emetine solutions containing 0·2 × 10^{-3}; 0·2 × 10^{-4} and 0·1 × 10^{-4}mmol ml^{-1} emetine so that the total amount infused in each case was 3·0, 0·3 and 0·15 mg. All of the concentrations of emetine examined significantly reduced the uptake of ^{14}C-NA by the heart (Fig. 1). The degree of reduction appeared proportional to the concentration used.

Emetine concentrations of 0·2 × 10^{-3} and 0·2 × 10^{-4}mmol ml^{-1} arrested the hearts in diastole soon after the initiation of perfusion. This effect was rapidly reversed by perfusion with emetine-free Krebs-Ringer solution. A concentration of 0·1 × 10^{-4}mmol ml^{-1} did not affect heart rate after 6 min of perfusion (control 180 ± 22; emetine treated 181 ± 25, n = 6) but significantly reduced uptake of ^{14}C-NA (Fig. 1). These data indicate that the ability of emetine to prevent the uptake of noradrenaline is not related to its effect on heart rate.

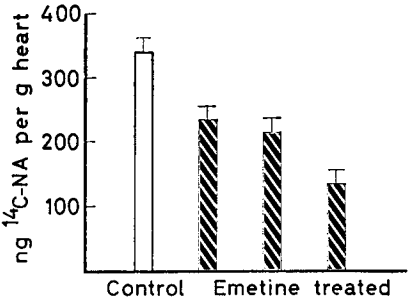

FIG. 1. The effect of emetine on the 6 min uptake of ^{14}C-NA (100 ng ml^{-1}) by the heart. From left to right: control values, $0{\cdot}2 \times 10^{-3}$, $0{\cdot}2 \times 10^{-4}$ and $0{\cdot}1 \times 10^{-4}$mmol emetine ml^{-1} of perfusate. Each column represents the mean $\pm$ the standard deviation. All doses of emetine significantly reduced uptake ($P < 0{\cdot}01$, n = 6).

Perfusion with emetine solution ($0{\cdot}2 \times 10^{-3}$mmol ml^{-1}) for 10 and 20 min did not significantly affect the release of ^{14}C-NA (10 min control 206 $\pm$ 36; 10 min emetine 193 $\pm$ 34; 20 min control 141 $\pm$ 50; 20 min emetine 101 $\pm$ 44; n = 6). However, after 60 min perfusion emetine-treated hearts contained significantly less ^{14}C-NA than control hearts (ng g^{-1}, emetine-treated 250 $\pm$ 47; control 389 $\pm$ 53 n = 6). These observations suggest that the primary effect of emetine is on the re-uptake rather than the release of noradrenaline.

Both emetine and guanethidine, a drug similar to emetine in its pharmacological properties, significantly reduced the *in vivo* myocardial concentration of ^{14}C-NA [control 162$\pm$15 ; emetine (1 mg kg^{-1}, i.p.) 106$\pm$10; guanethidine (0·486 mg kg^{-1} i.p.) 114 $\pm$20 n = 6] Saline-treated animals showed no significant differences in myocardial ^{14}C-NA concentrations from control values.

DISCUSSION

^{14}C-NA has been demonstrated to be a convenient and reliable method for studying the effects of various agents on the metabolism of noradrenaline (Axelrod, 1968). Most of the infused ^{14}C-NA retained by cardiac tissues enters the cardiac sympathetic neurons, mixes with endogenous noradrenaline and is subsequently metabolized in much the same fashion as the endogenous noradrenaline.

In this study the inclusion of emetine in the perfusing medium reduced the myocardial uptake of noradrenaline in the rat isolated perfused heart (Fig. 1). The degree of reduction was proportional to the concentration of emetine used and related to the sequence in which noradrenaline and emetine were perfused. The results suggest that emetine blocks the direct uptake of noradrenaline by the sympathetic neurons in the heart and that if noradrenaline is first permitted to enter the neuronal cells the effect of emetine is then dependent upon the subsequent release and re-uptake processes.

It is unlikely that emetine reduced the uptake of noradrenaline through action on heart rate since a low concentration of emetine ($0{\cdot}1 \times 10^{-4}$mmol ml^{-1}) which had no effect on heart rate significantly reduced the 6 min uptake of noradrenaline (Fig. 1).

Emetine treatment (1 mg kg^{-1}) was shown to decrease *in vivo* myocardial concentrations of noradrenaline 90 min after intraperitoneal injection. Guanethidine, in an equimolar concentration, also reduced *in vivo* myocardial concentrations of

noradrenaline. The latter observation is in agreement with previous reports (Cass & Spriggs, 1961; Fielden & Green, 1967). Guanethidine was chosen for comparison with emetine because it is considered to be representative of drugs that depress postganglionic adrenergic transmission (Goodman & Gilman, 1970). Guanethidine apparently blocks adrenergic function by impairing the passage of postganglionic nerve impulses which is followed secondarily by a decline in the tissue concentrations of noradrenaline (Rand & Trinker, 1966). In addition, chronic administration of guanethidine renders effector cells supersensitive to noradrenaline in a similar manner to that seen after sympathetic post-ganglionic denervation (Goodman & Gilman, 1970). Reports have shown that emetine acts like an adrenergic neuron blocking agent (Ng, 1966; Salako, 1970). The results of this study indicate that clinical doses of emetine can block the uptake of noradrenaline *in vivo* and *in vitro*. This observation suggests the possibility that the increase in cardiac arrhythmia and irritability seen during emetine treatment may be due to its effect on endogenous noradrenaline stores causing an increase in both the level of circulating catecholamines and the sensitivity of myocardial tissue.

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