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The effect of pancuronium on myocardial contraction and catecholamine metabolism

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The effects of pancuronium bromide infusion on the uptake and release of [^{14}C] noradrenaline (^{14}C-NA) by the isolated, perfused rat heart and on the chronotropic and inotropic activity of the isolated heart were evaluated. 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 pancuronium on the uptake of noradrenaline was determined by perfusing hearts for 5 min with perfusate containing various concentrations of pancuronium and 200 ng ml^{-1} of ^{14}C-NA. After 5 min pancuronium-treated hearts contained less ^{14}C-NA. The degree of reduced uptake increased with increasing concentrations of pancuronium. In addition, the combination of pancuronium perfusion and electrical stimulation (15 mA for 10 ms at 4 Hz) blocked the 50 min uptake of ^{14}C-NA by the heart to a greater degree than either factor separately. The release of noradrenaline was determined after perfusing hearts with ^{14}C-NA followed by perfusion with solution containing pancuronium but no ^{14}C-NA for 1 h. Pancuronium infusion did not significantly alter the release of ^{14}C-NA from the heart after 1 h of perfusion. The infusion of pancuronium caused a reduction in both the rate and strength of myocardial contraction of the isolated heart which was reversed by perfusion with perfusate free of pancuronium. Following perfusion with pancurnium the rate and strength of contraction of the heart was seen to "rebound" above pre-pancuronium values for a short period. The rebound of myocardial rate and contraction may have been due to the presence of myocardial noradrenaline previously blocked from reuptake by pancuronium since hearts removed from reserpinized animals did not demonstrate "rebound."

Pancuronium, a steriodal, non-depolarizing neuromuscular blocking agent, has been shown to transiently elevate heart rate and blood pressure immediately after administration to both man and animals (Loh, 1970; Smith, Proctor & Spence, 1970; Kelman & Kennedy, 1971). While this effect is generally moderate during general anaesthesia, episodes of substantially increased cardiac output have been noted (Gertel, Fox & others, 1972). If pancuronium possesses sympathomimetic acitvity it would be expected that increases in blood catecholamines could be demonstrated following its application. However, results from reports conflict on this matter. Nana, Cordan & Domokos, (1973) have shown that blood catecholamine concentrations are elevated after pancuronium during halothane anaesthesia in man, while Zsigmond, Matsuki & others, (1974) have found no significant change in plasma noradrenaline after pancuronium administration during thiamylal-N_2O anaesthesia.

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No doubt the difficulty in assaying blood catecholamines may have contributed to this conflict of results particularly if the ability of pancuronium to elevate blood borne catecholamines is moderate. We have measured directly the effects of pancuronium on the turnover rate of radioactively labelled noradrenaline in the isolated, perfused rat heart. In addition, since the sympathomimetic effects of pancuronium may be potentiated by heightened sympathetic activity, the combined effects of electrical stimulation and pancuronium infusion on the uptake of ^{14}C-NA by the isolated heart were evaluated.

METHODS

The hearts were quickly removed from male, Sprague-Dawley rats, 200–250 g, under light ether anaesthesia, washed in cold saline solution and perfused with a bilateral perfusion pump at a constant rate of 5 ml min^{-1} and a pressure of approximately 40 mm Hg as described by Miletich, Ivankovich & others (1974). The perfusate media was Krebs-Ringer with bicarbonate containing in addition (per litre) glucose 1 g, ascorbic acid 20 mg, and ethylenediamine-tetracetic acid disodium (EDTA) 10 mg. The perfusates were gassed with 5% CO_2 in oxygen. In each experiment the control and test hearts were perfused for 5 to 10 min with normal 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) (New England Nuclear, specific activity: 55·3 m Ci mmol^{-1}) were determined by perfusing the hearts with Krebs-Ringer containing 200 ng ml^{-1} of ^{14}C-NA for 5 min. The hearts were then perfused for 90 s with ^{14}C-NA 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. Uptake values for experimental hearts were determined in a similar fashion except that test doses of pancuronium bromide (Pavulon, Organon) were added to the perfusate.

The 1 h release of ^{14}C-NA from control and pancuronium-treated hearts was determined by first perfusing each heart with Krebs-Ringer solution containing 200 ng ml^{-1} ^{14}C-NA for 10 min after which the hearts were perfused with Krebs-Ringer with or without pancuronium (13·6 n mol ml^{-1}) for 1 h. 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 effect of electrical stimulation on the 50 min uptake of ^{14}C-NA by control and pancuronium-treated hearts was determined by pacing the hearts with a rectangular dc pulse generator while being perfused with perfusate containing 50 ng ^{14}C-NA ml^{-1}. A pulse of 15 mA at 4 Hz of 10 ms duration was found to be most effective in reducing ^{14}C-NA uptake and was therefore used.

The effects of pancuronium on myocardial contractile force and spontaneous rate of the isolated heart was measured by attaching a Hewlett-Packard linear motion transducer (model # FTA-10-1) via a four inch silk thread sutured to the apex of the heart muscle. The perfusate temperature was reduced to 32° since the contractility of the preparation remained more stable at this temperature. In addition, the effect of pancuronium perfusion on myocardial contractility was evaluated in hearts from rats which had been previously administered reserpine intraperitoneally (1·0 mg kg^{-1}) 48, 24 and 2 h before removal of the hearts.

RESULTS

The effect of perfusing hearts with perfusates containing pancuronium on the 5 min uptake of ^{14}C-NA can be seen in Fig. 1. Dose rates of 680 and 68 n mol min^{-1} significantly reduced the uptake of ^{14}C-NA when compared with control values. The reductions in uptake of ^{14}C-NA by the lower dose rates 6·8 n mol and 0·68 n mol min^{-1} were not statistically significant but suggested that the degree of uptake impairment was proportional to the dosage of pancuronium employed.

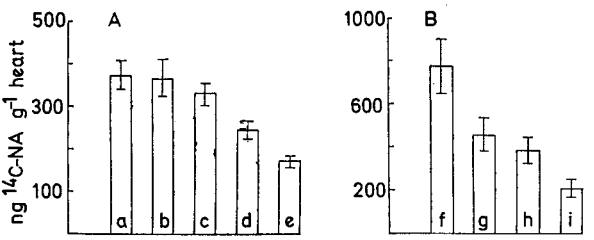

FIG. 1A. The effect of pancuronium bromide infusion on the 5 min uptake of ^{14}C-NA (200 ng ml^{-1}) by the isolated heart. Control (a) dose rates of 0·68 n mol min^{-1} (0·136 n mol ml^{-1}) (b) and 6·8 n mol min^{-1} (1·36 n mol ml^{-1}) (c) were not significantly different from control; 68 n mol min^{-1} (13·6 n mol ml^{-1}) (d) and 680 n mol min^{-1} (136 n mol ml^{-1}) (e) significantly blocked ^{14}C-NA uptake ($P \leqslant 0{\cdot}01$, n = 6 for each group).

B. The effect of electrical stimulation (15mA for 10 ms at 4 Hz) and pancuronium bromide (680 n mol min^{-1}) on the 50 min uptake of ^{14}C-NA (50ng ml^{-1}) by the isolated heart. Control (f) electrical stimulation (g) pancuronium (h). The combination of electrical stimulation and pancuronium infusion (i) significantly reduced the uptake of ^{14}C-NA to a greater extent than either factor separately ($P \leqslant 0{\cdot}02$, n = 6 for each group).

Pancuronium infusion had little effect on the release of ^{14}C-NA from the isolated heart. No significant reductions in myocardial concentrations of ^{14}C-NA were seen after 1 h of perfusion at a dose rate of 680 n mol min^{-1} (ng g^{-1} control 343 $\pm$ 49; pancuronium treated 325 $\pm$ 31; n = 6). This observation suggests that the primary effect of pancuronium is on the membrane uptake process for noradrenaline and does not interfere with the storage or release processes.

A square wave electric impulse of 15 mA applied at 4 Hz of 10 ms duration each significantly reduced the 50 min uptake of ^{14}C-NA (Fig. 2). Inclusion of pancuronium (dose rate: 680 n mol min^{-1}) in the perfusate significantly enhanced the electrically induced reduction in ^{14}C-NA uptake.

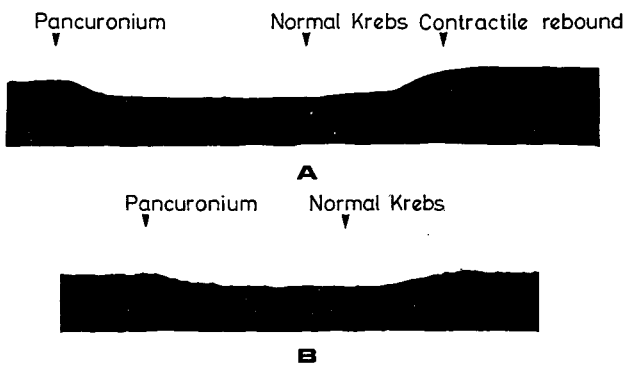

FIG. 2.A. The effect of pancuronium bromide (680 n mol min^{-1}) on the strength of contraction and spontaneous rate of the isolated heart. Both parameters were reduced by pancuronium but rebounded above pre-pancuronium values after returning to normal Krebs perfusion.

B. The effect of pancuronium bromide (680 n mol min^{-1}) on the strength and rate of contraction of hearts removed from reserpinized animals. Paper speed 1 mm s^{-1} or 2·5 mm s^{-1}.

The effect of pancuronium on the inotropic and chronotropic activity of the isolated heart can be seen in Fig. 2A. Pancuronium significantly reduced both the rate and strength of myocardial contraction (Table 1). This effect was reversible since infusion with perfusate free of pancuronium restored both the rate and strength of heart contractures to control values and in most instances these values rose substantially above pre-pancuronium levels. The rebound of these parameters above control values may have been due to the build up of extracellular myocardial noradrenaline during the pancuronium infusion period since prior reserpinization abolished this (Fig. 2B).

Table 1. *The effect of pancuronium infusion on the strength of contraction and spontaneous rate of the rat isolated heart.*

	% Decrease in strength of contraction	Heart rate before pancuronium infusion	Heart rate during pancuronium infusion
Pancuronium 680 n mol min^{-1} n = 6	36±15	180±24	114±32 P≤0·05
Pancuronium 68 n mol min^{-1} n = 10	13±8	172 ± 20	163 ± 12 n.s.

DISCUSSION

It is apparent that pancuronium infusion at dose rates of 680 and 68 n mol min^{-1} blocked the uptake of ^{14}C-NA by the isolated heart but a dose rate of 680 n mol min^{-1} had no significant effect on the 1 h release of ^{14}C-NA from the heart. These observations suggest that pancuronium acts primarily on the neuronal membrane interfering with the uptake mechanism for noradrenaline but does not penetrate the cell to cause release of noradrenaline.

Application of low intensity short duration electrical stimuli to isolated organs has been shown to produce graded release of noradrenaline (Blinks, 1966; Katz & Kopin, 1969; Hiott & Richardson, 1971). Presumably, such electrical stimuli cause the release of noradrenaline in analogous fashion to release triggered by the nerve action potention. Drugs which block uptake or cause the release of noradrenaline have been demonstrated to increase the amount of noradrenaline released by electrical stimulation (Brown & Gillespie, 1957; Gillespie & Kirpekar, 1965). In this study the combination of pancuronium infusion and electrical stimulation resulted in a greater reduction in the uptake of ^{14}C-NA by the heart than either pancuronium or electrical stimulation separately (Fig. 1B). This observation may be interpreted to mean that in situations of heightened sympathetic activity the sympathomimetic effects of pancuronium might be potentiated.

Pancuronium, when applied to the isolated heart, at a dose rate of 680 n mol min^{-1}, produced a significant decrease in contractile force and in spontaneous rate (Table 1). This was followed by a rebound in contractile force above control values after returning to perfusion with perfusate free of pancuronium (Fig. 2A). A dose rate of 68 n mol min^{-1} resulted in a moderate decrease in contractile force with little change in rate. Myocardial depression by pancuronium may appear to be in contradiction to its

sympathomimetic-like action on the uptake of noradrenaline. However, it has been demonstrated that pancuronium causes the release of acetylcholine from the isolated gastrocnemius muscle of the frog (Gergis, Dretchen & others, 1972). Acetylcholine released by pancuronium in the heart could explain the reduction in contractile force and rate seen in this study providing that the amount of acetylcholine released prevailed over the amount of noradrenaline blocked from reuptake. The rebound in contractile force following pancuronium infusion above pre-pancuronium control values may have been due to the presence of extracellular, myocardial noradrenaline previously blocked from reuptake by pancuronium (Fig. 2A). In support of this possibility is the observation that hearts taken from reserpinized rats did not demonstrate contractile rebound after pancuronium infusion (Fig. 2B). The importance of myocardial catecholamines to ventricular contractile rebound has been shown in reserpinized dogs after coronary artery occlusion (Newman, Pershotam & Walton, 1971).

From the data presented in this study it would appear that pancuronium posseses sympathomimetic activity which could be potentiated during situations of elevated adrenergic discharge. The sympathomimetic action of pancuronium appears to be manifested primarily via inhibition of the uptake of noradrenaline by adrenergic nerve endings and therefore may explain the increase in heart rate and blood pressure seen immediately after its administration to man.

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