Influence of Perfusion Time on Norepinephrine Uptake, Heart Rate and Intracellular Cations in Guinea Pig Hearts[1,2] (36962)

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The isolated perfused heart has served as an extremely useful model for studying the uptake process which transports the adrenergic neurotransmitter across the axonal membrane. In addition, this preparation has been very valuable in studying the influence of pharmacological agents on this uptake process. By measuring arterio–venous (A–V) differences, it has been most advantageous in actually differentiating between the initial neuronal uptake and subsequent retention of the transmitter (1).

It has been reported that when certain isolated tissues such as the aorta are removed from an animal and placed in a bath of Krebs solution or plasma there is a marked movement of ions with potassium leaving and sodium entering the cell (2). These ions tend to return to normal shortly after being placed in this isolated environment. When these investigators minimized the handling of the tissue by perfusion of the aorta with Krebs solution, however, they did not observe this very rapid exchange of electrolytes. They did see a gradual loss of potassium and an increase in sodium over a 90-min perfusion period.

The purposes of the present experiments were: (a) to see what influence perfusing the guinea pig heart for various periods of time would have on intracellular electrolyte levels; (b) to see if there were any changes in the ability of the heart to take up norepinephrine after being perfused for various periods of time; this seemed of particular importance because of the dependency of amine uptake on extracellular sodium; and (c) to determine if there were any differences in the sensitivity of the myocardial pacemaker to isoproterenol and/or norepinephrine as a result of varying the time of perfusing the heart prior to exposure to drugs.

Methods. Hearts were removed from male guinea pigs weighing between 200–400 g under pentobarbital anesthesia and immediately connected to an Anderson-Craver coronary perfusion apparatus via the aorta. The normal perfusion medium contained the following composition in mmole/liter: NaCl, 119.8; KCl, 5.63; $CaCl_2$, 2.16; $MgCl_2$, 2.10; dextrose, 10.0 and $NaHCO_3$, 25.0. The solution was bubbled with 95% O_2–5% CO_2; the temperature was maintained at 38 ± 1° and pH 7.32–7.4. All hearts were perfused at a constant flow of 6.0 ml/min by means of a Harvard infusion pump. Four groups of hearts were perfused for the following studies: (a) uptake of norepinephrine, (b) inulin space, (c) electrolytes, and (d) dose response curves.

Uptake studies. Hearts were perfused with normal medium for 5, 30, 60, or 120 min and then switched to a medium containing 2 ng/ml dl/—7 ^3H norepinephrine and 8 ng/ml l-norepinephrine. Ascorbic acid (10 μg/ml) and EDTA (10 ng/liter) were added to the perfusion medium to prevent the oxidation of norepinephrine. Hearts were perfused for 10 min with norepinephrine and then switched back to a medium devoid of amine for an additional 3 min. Samples were collected at 1-min intervals during the norepinephrine perfusion in ice cold tubes containing 5 mg ascorbic acid. Aliquots from each tube were immediately taken for scintillation spectrometry as well as for chemical analysis utilizing the

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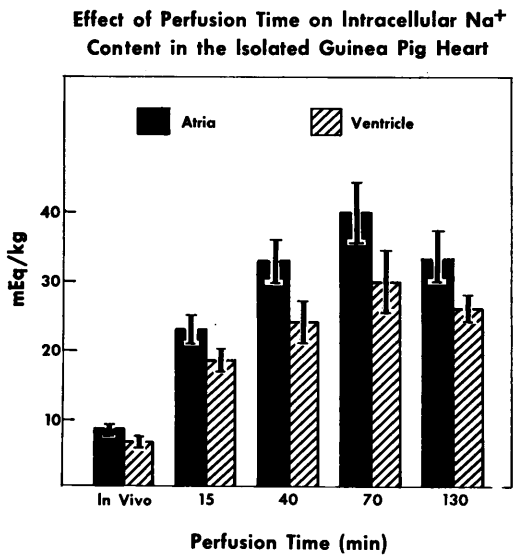

FIG. 1. The effect of altering the perfusion time on the intracellular sodium concentration. Data are plotted as mEq/kg dry weight ± standard error of the mean vs time in minutes. Black bars represent atria and dashed bars ventricle. Each bar represents mean data from at least 15 animals.

automated trihydroxyindole method (3). In addition to measurements of A–V norepinephrine differences, the hearts were removed at the end of the perfusion, weighed, homogenized in 5% trichloroacetic acid, filtered under suction and the clear filtrates adsorbed on alumina columns according to the method of Euler and Lishajko (4).

An aliquot of the alumina column tissue eluates was used for radioactive analysis utilizing liquid scintillation spectrometry, another for fluorometric analysis utilizing the automated trihydroxyindole method and a final aliquot taken for analysis of deaminated metabolites using the ethyl acetate method (5).

Inulin space determination. Extracellular space in the perfused hearts was measured by utilizing ^{3}H-methoxyinulin. Hearts were perfused for various periods of time with normal medium and then perfused with ^{3}H-inulin for 15 min. Hearts were removed, blotted gently, weighed, homogenized in 5% TCA and an aliquot of the supernatant taken for liquid scintillation spectrometry. Inulin space was calculated by dividing dpm/g (wet weight of heart) by dpm/ml in the perfusion medium.

Electrolyte determination. Hearts were perfused for various periods of time and then the entire atria and a sample of ventricle taken for electrolyte determinations. The tissues were blotted gently, weighed (wet weight), placed in pre-weighed platinum crucibles and dried in an oven at 98° for 20 hr. The samples were reweighed and gradually taken up to 540° (11 hr) for ashing in a furnace. One ml of 1 *N* HCl was added to each sample dissolving the ash and the Na^{+}, K^{+}, Ca^{++}, and Mg^{++} concentrations measured by atomic adsorption spectrometry. (Jarrell-Ash-Dial Atom 11).

Dose–response curves. A silk thread was tied to the apex of the ventricle, passed over a nylon pulley and connected to a Grass force displacement transducer. Heart rate was recorded on a Brush (Mark 220) recorder.

Dose response curves were carried out with dl-isoproterenol and l-norepinephrine (Winthrop Laboratories) at various periods of time of perfusion. The volume of drug (0.5 ml) was added via a side arm to the perfu-

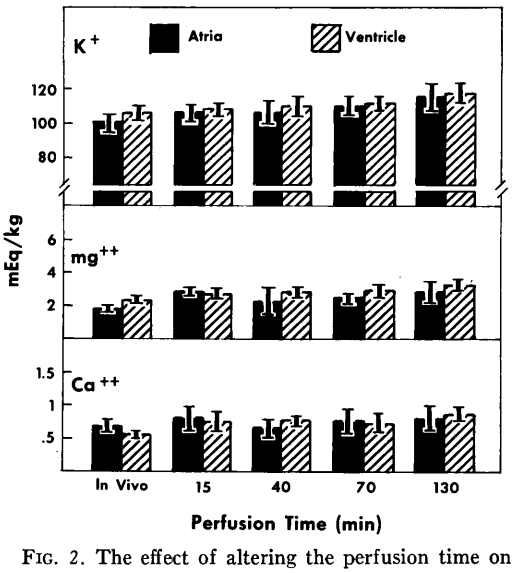

FIG. 2. The effect of altering the perfusion time on the intracellular potassium, magnesium, and calcium in the atria and ventricle. Data are plotted as mEq/dry weight ± standard error of the mean vs time in minutes. Each bar represents mean data from at least 15 animals.

TABLE I. Effect of Perfusion Time on Total and Cellular Water in Atria from Isolated Guinea Pig Hearts.

Total time of perfusion (min)	Total water (ml/kg) ± S.E.M. (p)[a]	Cell water (ml/kg) ± S.E.M. (p)[a]
In Vivo	795 ± 11.5	430 ± 11.7
15	851 ± 13.7 ($p < .01$)	486 ± 13.7 ($p < .025$)
40	864 ± 16.7 ($p < .01$)	449 ± 9.9 (NS)
70	849 ± 13.2 ($p < .025$)	489 ± 15.8 ($p < .025$)
130	879 ± 9.5 ($p < .001$)	499 ± 14.4 ($p < .005$)

[a] Compared to *in vivo* value.

sion cannula and washed in with 1.5 ml of warm perfusion medium. Doses of drugs were added at 5-min intervals with each dose response curve taking 35 min. Ascorbic acid (100 μg/ml) and sodium metabisulfite (30 mg) were added to prevent oxidation of the catecholamines. Concentration of drugs used was as the free base.

Statistical analysis of data was by Student's t test.

Results. Figure 1 depicts the effect of altering the length of perfusion time on the intracellular Na^+ concentration of the atria and ventricles. There was a marked increase in the Na^+ concentration at 15 min which reached a peak after 70 min of perfusion. A similar pattern was observed for both tissues. The effect of varying the perfusion time on the intracellular content of other ions is shown in Fig. 2. Contrary to what was observed for intracellular Na^+, there were no significant alterations in K^+, Mg^{++}, or Ca^{++}.

The total water and all water content in the atria and ventricle was also determined (Tables I and II). In both cases there was a significant increase in the total water and cell water which was already elevated by 15 min of perfusion. Neither total or cell water increased further upon continued perfusion of the heart for longer periods of time.

Figure 3 shows the uptake and retention of norepinephrine carried out after the hearts had been perfused with normal medium for various lengths of time. The results indicate that the greatest uptake occurred in hearts that had been perfused with normal medium for only 5 min prior to the addition of ^{3}H-norepinephrine to the perfusion fluid. When ^{3}H-norepinephrine accumulation by the hearts was measured after perfusion with normal medium for 30 min, there was less uptake with the least uptake occurring after 60 min of pre-perfusion with normal medium. There was a greater uptake of amine when tested after the hearts had been pre-perfused for 120 min as compared to 60 min.

The washout of ^{3}H-norepinephrine from the heart following a 10-min perfusion with labeled amine did not vary as a function of how long the heart had been perfused prior to the addition of ^{3}H-norepinephrine to the perfusion medium. Also, there were no significant differences in the endogenous norepinephrine levels in hearts perfused for different periods of time. The norepinephrine content in hearts perfused for 5, 30, 60, or 120 min was 1.53 ± 0.32; 1.43 ± 0.29; 1.36 ± 0.26 and 1.22 ± 0.25, respectively. In addition, there were no significant differences in the ^{3}H-deaminated metabolites present in the heart when determined after the hearts had been perfused for various lengths of time prior to perfusion with ^{3}H-norepinephrine.

The responsiveness of the cardiac pacemaker as analyzed by dose–response curves to dl-isoproterenol is demonstrated by Fig. 4. It can be seen that the dose–response curve carried out after the heart had been perfused for 30 min was significantly shifted to the left as compared to curves carried out after 15- or 60-min of perfusion. This shift is statistically significant at the level of the ED50. Contrary to these curves, similar dose–response curves to l-norepinephrine (Fig. 5) did not show any significant differences when carried out after the hearts had been perfused for various lengths of time.

Discussion. In recent years the uptake of norepinephrine across the axonal membrane of adrenergically innervated tissues has been well characterized. It has been shown to: proceed against a considerable concentration gradient (6–8); obey saturation kinetics of the Michaelis-Menten type (8, 9); be inhibited by metabolic poisons (10–12); be tem-

TABLE II. Effect of Perfusion Time on Total and Cellular Water in Ventricle from Isolated Guinea Pig Hearts.

Total time of perfusion (min)	Total water (ml/kg) ± S.E.M. (p)[a]	Cell water (ml/kg) ± S.E.M. (p)[a]
In Vivo	805 ± 2.7	440 ± 2.7
15	850 ± 8.8 ($p < .001$)	485 ± 8.9 ($p < .005$)
40	844 ± 6.5 ($p < .001$)	452 ± 6.5 (NS)
70	851 ± 3.7 ($p < .005$)	487 ± 11.9 ($p < .001$)
130	878 ± 13.1 ($p < .001$)	505 ± 49 ($p < .001$)

[a] Compared to *in vivo* value.

perature dependent (1); show an absolute requirement for extracellular sodium (11, 13–15).

Since many isolated tissues show a marked alteration in intracellular Na^+ and K^+ levels when placed in an isolated environment, it was thought that a similar phenomenon might also occur in the perfused heart. This preparation has been frequently used as a model to study the uptake of norepinephrine. If alterations in Na^+ or K^+ levels do occur during the perfusion of the heart, this might influence the uptake of norepinephrine. The purpose of the present studies, therefore, was to measure the Na^+ and K^+ levels as a function of the perfusion time and to correlate these ions with the uptake of norepinephrine. An additional purpose was to see if there were any changes in the sensitivity of the receptor. This information has obvious implications concerning studies on the effect of drugs on uptake of norepinephrine as well as adrenergic agonists and antagonists. The data have demonstrated that there are, in fact, marked alterations in intracellular Na^+ concentrations, the uptake of norepinephrine and the responsiveness to isoproterenol as a function of altering the perfusion time.

Previously, several investigators have reported changes in the Na^+ and K^+ levels when tissues are placed in an isolated environment (16). For instance, it was observed that when rat aorta was excised and placed in a bath of Krebs solution, it lost over two-thirds of its potassium and gained roughly an equivalent amount of sodium within the first 15 sec (2). However, these rapid changes in aorta electrolyte composition did not occur when the rat was perfused with Krebs solution by intravenous infusion. Under these conditions over a 90-min perfusion period, there were changes noted with a decrease in potassium and increase in sodium. In the present studies we have observed a similar phenomenon involving sodium, with maximal increase in intracellular sodium after 70 min of perfusion. This was followed by a return of the sodium towards normal values. Unlike Dawkins and Bohr (2), we observed no significant difference in myocardial intracellular potassium.

It is well documented that the uptake of norepinephrine across the axonal membrane is dependent upon extracellular sodium (11, 13–15). In the present study, it was observed that there was a decrease in the uptake of norepinephrine which paralleled an increase in intracellular sodium. When the sodium began to return towards normal, there was also a greater uptake of norepinephrine (Figs. 1 and 3).

The fact that there was no difference in

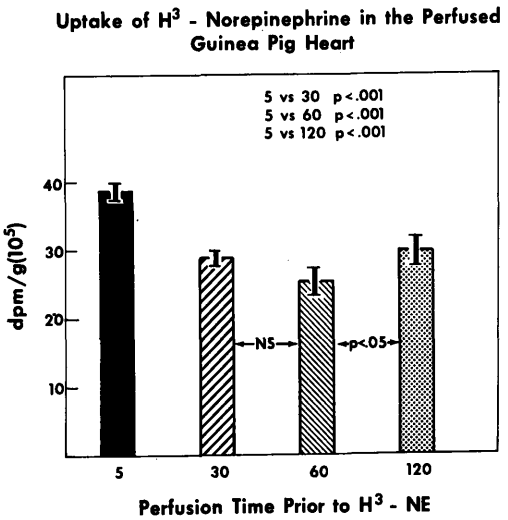

FIG. 3. Uptake of H^3-norepinephrine in the perfused heart after hearts had been perfused for various periods of time prior to perfusing with ^3H-norepinephrine. Data represents uptake obtained from tissue analysis in dpm/g × 10^5 ± standard error of the mean vs time in min.

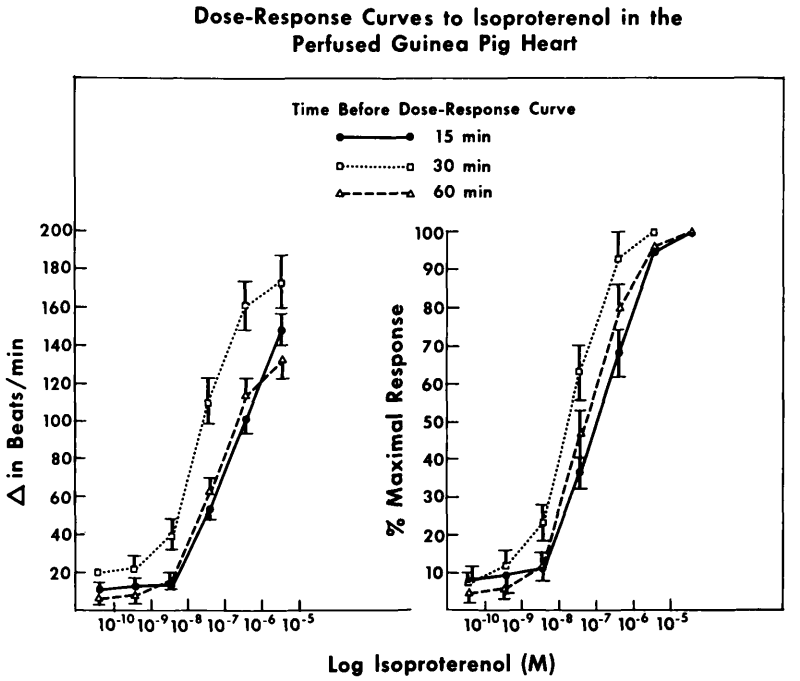

FIG. 4. Dose–response curves to l-isoproterenol in hearts perfused for 15, 30, or 60 min prior to starting dose response curve. Left panel shows change in beats/min and panel to the right % of maximal response to *M* concentrations of l-isoproterenol. Each point represents the mean of at least 6 hearts.

the deaminated catecholamine metabolites in the heart supports the idea that there was in fact a decrease in initial uptake across the axonal membrane rather than a deficiency in storage leading to increased deamination by monoamine oxidase.

To test whether or not there was any change in the sensitivity of the adrenergic receptor as a result of altering the length of perfusion time, dose–response curves were constructed for isoproterenol and norepinephrine. Data obtained with isoproterenol indicated that there is a leftward shift of the dose–response curve when hearts had been perfused for 30 min prior to constructing the curve. This was significant at the level of the ED_{50}. When the hearts were perfused for a longer period of time, the dose–response curve was more like the one seen after 15 min of pre-perfusion. This phenomenon also seemed to follow the pattern seen with intracellular sodium and uptake of norepinephrine in that when the intracellular sodium was highest and uptake of norepinephrine lowest, the receptors for isoproterenol were most sensitive. Since isoproterenol is not inactivated via neuronal uptake, this response could represent increased receptor sensitivity. The data obtained with norepinephrine, however, did not indicate any significant shifts in the dose–response curve. This result was unexpected because it was thought that if there was a decrease in the uptake of norepinephrine into neuronal sites, there would be more amine available to stimulate the receptor and a potentiation or supersensitivity should ensue. It is possible that the norepinephrine was taken up into extraneuronal tissue or there was an increase in O-methylation. This would account for the fact that no potentiation was observed in spite of decreased neuronal uptake of norepinephrine. Further experiments are necessary to determine if this is, in fact, the case. Because of the different results obtained with isoproterenol compared to norepinephrine, no definite conclusions concerning increased receptor sensitivity can be made as a result of this present study.

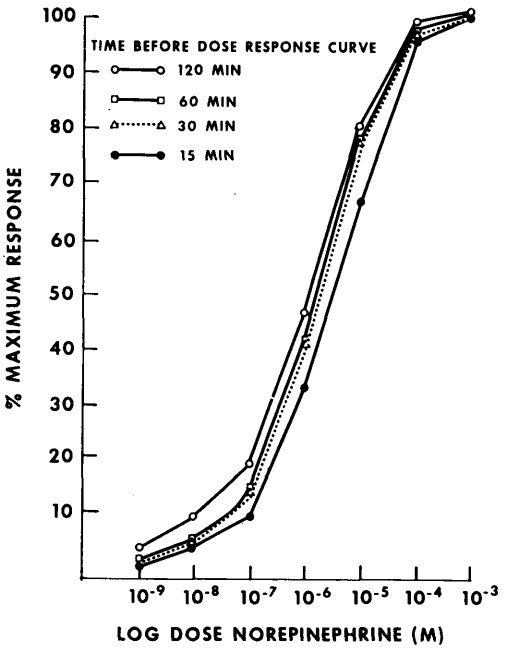

FIG. 5. Dose response curves to l-norepinephrine in hearts perfused for 15, 30, 60, or 120 min prior to starting the dose–response curve. Data are plotted as % maximal response vs *M* concentrations of l-norepinephrine. Each point represents the mean of at least 6 hearts.

Summary. The present study has shown that there is a deficiency in the ability of an adrenergic neurone to take up norepinephrine for a period of time after removing the heart from the guinea pig. An increase in intracellular sodium parallels the decrease in uptake. There is a return toward the normal environment upon continued perfusion. These studies have obvious implications in investigations carried out on the effect of drugs and other perturbations on the uptake of norepinephrine.

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