up to 30 days after surgery. The kallikrein excretion of 15 and 30 days UNx rats was lower than C even when expressed per g of kidney weight. Only 15 days UNx rats had diminished RKal. UNx rats had normal blood pressure (all groups), increased urine vol. (15 and 30 days), increased natriuresis (30 days) and decreased negative free water clearance (15 days). UKalV from all animals was inversely related to sodium excretion, Na^+/K^+ ratio, as well as to urine vol., and directly related to the negative free water clearance.

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Department of Pharmacology
University of Heidelberg
Im Neuenheimer Feld 366
D-6900 Heidelberg, F.R.G.

MARCOS MARIN-GREZ*
GASTON SCHAECHTELIN

* To whom reprint requests should be addressed.

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Uptake of [^{3}H]nitrendipine into cardiac and smooth muscles

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Nitrendipine, an asymmetrically substituted derivative of 1,4-dihydropyridine, is a new cardiovascular agent belonging to the class of calcium antagonists [1]. This drug causes coronary vasodilation and exerts an antihypertensive action [2].

The present study was undertaken to probe further the mechanism of action of nitrendipine, in particular its ability to cross the surface membrane of the muscle cells. The uptake of nitrendipine by cat ileal smooth muscle and by chick embryonic ventricular muscle was examined.

Cat ileal smooth muscle strips were prepared according to the method of Sperelakis [3]. A long segment of ileal small intestine was removed from anesthetized cat and immediately immersed in ice-cold Ringer solution. The lumen of the cat intestine was washed thoroughly with ice-cold Ringer solution using a syringe. A segment (3–4 cm) of intestine was mounted on a 5-ml pipet for dissection. The outer serosa and the longitudinal muscle layer were carefully removed with a pair of fine forceps. Then, the intestinal tube was inverted and remounted on the pipet. The layer of mucosa and submucosa was carefully separated from the circular muscle layer and stripped off. The remaining muscle tube contained relatively pure circular smooth muscle and was dissected into rings 5 mm wide, which were stored at 0° in Ringer solution before use.

Embryonic hearts were removed from fertilized chicken eggs (White Leghorn, Babcock strain) at 9 days of age. The ventricles were then dissected from the hearts, washed and stored in ice-cold Ringer solution before use.

The composition of the Ringer solution for [^{3}H]nitrendipine uptake was: 145 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 20 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes) (pH 7.4) and 10 mM glucose. Nitrendipine (Miles Laboratories) was dissolved in absolute ethanol and added to give final concentrations of 10^{-9}–10^{-6} M. Appropriate amounts of [^{3}H]nitrendipine (88 Ci/mmole, ICN Pharmaceuticals) were added.

The muscles were incubated at 37° and, at appropriate time intervals, the muscle samples were removed from the incubation medium and blotted dry. To wash drugs taken up in the interstitial fluid space of the muscle preparations, the muscles were incubated for 10 min in ice-cold Ringer solution [4]. The muscle samples were again blotted, weighed (for the smooth muscle only), and dissolved in 0.1 N NaOH at 60° overnight. The radioactivity of the solubilized muscle sample was determined by liquid scintillation counting, and the protein content was determined by the method of Lowry *et al.* [5].

For efflux experiments, ileal muscle was loaded with 10^{-6} M nitrendipine or verapamil (1 μCi/ml) for 3 hr [4]. The muscles were then removed, blotted, dried, and passed through a series of efflux tubes containing 1 ml of Ringer's solution. The muscle was moved sequentially from tube to tube at 0.25, 0.5, 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, and

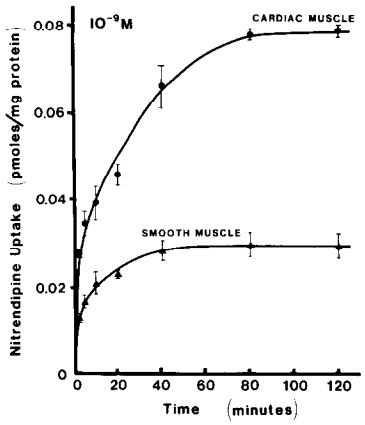

Fig. 1. Time course of uptake of nitrendipine into cat ileal smooth muscle and chick embryonic ventricular muscle (9-day-old). Uptake of nitrendipine was measured in the presence of 145 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10^{-9} M [^{3}H]nitrendipine, 20 mM Hepes, pH 7.4, and 10 mM glucose at 37°.

90 min after loading. Efflux solution was transferred into scintillation vials, and scintillation mixture was used to rinse the tubes two times. The ileal smooth muscles were then dissolved in 2 ml of 0.1 N NaOH at 60° overnight and treated as described previously.

The tissue/medium (T/M) ratio was expressed as the amount of nitrendipine taken up by the muscles at steady state, with respect to the concentration of nitrendipine in the medium (pmoles per g wet wt/pmoles per ml of medium). The conversion factor between the wet weight of the cardiac muscle and the mg protein content was determined in a separate series of experiments. The protein concentration was 92 ± 7 mg/g wet wt for chick ventricular muscle and 132 ± 7 mg/g wet wt for cat ileal smooth muscle. $[Nitrendipine]_i/[Nitrendipine]_o$ was calculated from the

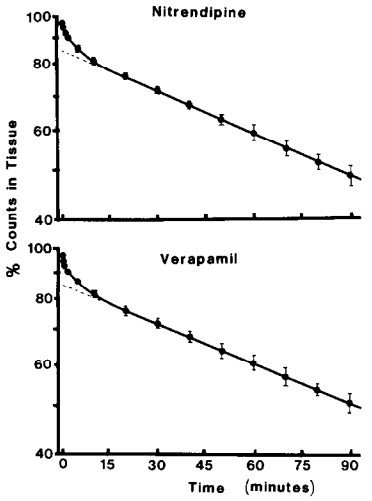

Fig. 2. Efflux of [^{3}H]nitrendipine and [^{3}H]verapamil from cat ileal smooth muscle. Ileal muscle was loaded for 3 hr in 10^{-6} M nitrendipine or verapamil containing trace amounts of [^{3}H]nitrendipine (1 μCi/ml) or [^{3}H]verapamil (1 μCi/ml) and then effluxed into Ringer solution at 37°. Two compartments were observed, probably reflecting efflux from the intracellular (slow) and extracellular (fast) space.

T/M ratio after correction for: (a) the water content being 80% of the total wet weight, (b) the interstitial space being about 15% of the muscles, and (c) the loss of radioactive label from the slow (presumably intracellular) compartment during the 10-min wash period of about 6% (see Fig. 2).

Figure 1 shows the time course of uptake of nitrendipine into cat ileal smooth muscle and chick embryonic ventricular muscle. The uptake of [^{3}H]nitrendipine reached maximum at 40 min (smooth muscle) and 80 min (cardiac muscle). At equilibrium, the influx of labeled nitrendipine should be equal to the efflux of labeled nitrendipine.

Figure 2 shows the efflux of nitrendipine from cat ileal smooth muscle. The efflux curve is made up of two compartments. The half-time for efflux of nitrendipine from the fast compartment was 2.81 ± 0.07 min. The half-time of the slow compartment was 110.4 ± 3.5 min. To confirm the efflux data on nitrendipine, the efflux of [^{3}H]verapamil was also measured. As shown in Fig. 2, the half-times for the fast compartment (3.22 ± 0.12 min) and the slow compartment (119.1 ± 4.7 min) were quite similar to those for nitrendipine. From these efflux experiments, it is apparent that a 10-min wash would essentially empty the fast compartment (presumably the extracellular space) without appreciably affecting the slow compartment (i.e. the intracellular space).

As indicated by the maximal nitrendipine uptake and T/M ratio (Table 1), chick ventricular muscle accumulated at least 2-fold more nitrendipine (10^{-9}–10^{-6} M) than cat smooth muscle. As shown in Table 2, cooling of the cat ileal smooth muscle to 27° and 17° did not greatly affect the influx rate, indicating that the uptake was not related to active transport process. However, cooling to 7° did produce a marked decrease in influx, perhaps due to change in the membrane fluidity. Calculation of the distribution of nitrendipine at steady state indicates that the nitrendipine concentration inside the muscle cells was much higher than the outside (Table 1).

The present study indicates that nitrendipine was accumulated by cat ileal smooth muscle and chick embryonic ventricular muscle. The uptake of nitrendipine can be considered to be distributed in at least three compartments of the muscles: (a) extracellular or interstitial space, (b) plasma membrane binding sites, and (c) intracellular space and binding sites. Since the muscles in this study had been washed after incubation in the calcium antagonist, we assume that the interstitial accumulation of the drug was washed out completely [4]. Thus, our data on uptake reflect the binding of nitrendipine to the plasma membrane and intracellular accumulation.

A high T/M ratio was found for nitrendipine in cat smooth muscle and chick ventricular muscle (Table 1). After correction for water content, interstitial space, and loss of radioisotope during the wash period, nitrendipine concentration inside the muscle cells was 4.67- to 6.48-fold greater than outside for the cat smooth muscle and 8.10- to 11.31-fold for chick ventricular muscle (Table 1).

The comparatively higher internal concentration of nitrendipine at steady state was not a result of active transport of nitrendipine into the muscle cells, since (a) there was no evidence of saturation kinetics in the nitrendipine concentration range of 10^{-9}–10^{-6} M (Table 1), and (b) there was no decrease in uptake when the incubation temperature was decreased from 37° to 17° (Table 2). The high internal concentration of nitrendipine could simply reflect passive diffusion down a concentration gradient, if the drug binds to many sites internally in the muscle cells. The internal binding sites could include sites on the sarcoplasmic reticulum (SR) membranes and sites on the mitochondrial membranes as, for example, it has been shown that calcium uptake into these organelles is depressed by other calcium antagonists, e.g. verapamil [6, 7]. Consistent with this possibility is the finding that the maximal number of nitrendipine binding sites was several fold higher in the microsomal fraction than in the whole homogenate of the rat

Table 1. Characteristics of nitrendipine uptake into cardiac and smooth muscles*

$[Nitrendipine]_o$	Initial rates (pmoles/mg/min)	$T_{\frac{1}{2}}$ (min)	Maximal uptake (pmoles/mg)	T/M ratio	$\frac{[Nitrendipine]_i}{[Nitrendipine]_o}$	$[Nitrendipine]_i$
(I) Cat ileal smooth muscle						
1×10^{-9} M	0.005 ± 0.000	5	0.03 ± 0.00	3.91	6.12	6.1×10^{-9} M
1×10^{-8} M	0.07 ± 0.00	4	0.31 ± 0.02	4.14	6.48	6.5×10^{-8} M
1×10^{-7} M	0.65 ± 0.02	4	2.90 ± 0.09	3.84	6.01	6.0×10^{-7} M
1×10^{-6} M	5.55 ± 0.37	3	22.64 ± 1.75	2.99	4.67	4.7×10^{-6} M
(II) Chick embryonic ventricular muscle						
1×10^{-9} M	0.01 ± 0.00	8	0.08 ± 0.01	7.23	11.31	1.1×10^{-8} M
1×10^{-8} M	0.11 ± 0.01	10	0.78 ± 0.04	7.15	11.19	1.1×10^{-7} M
1×10^{-7} M	1.09 ± 0.12	7	6.31 ± 0.37	5.80	9.07	9.1×10^{-7} M
1×10^{-6} M	6.74 ± 0.62	7	56.24 ± 4.75	5.17	8.10	8.1×10^{-6} M

* Initial rates were calculated from values obtained at 2.5 min of the uptake curves. Maximal uptakes were calculated from values at 80 and 120 min. $T_{\frac{1}{2}}$ was obtained graphically. T/M ratio was expressed as pmoles per g wet wt/pmoles per ml of medium. $[Nitrendipine]_i/[Nitrendipine]_o$ was calculated from the T/M ratio after correction (as described in text). Data are expressed as mean ± S.E.M. from six to eight experiments. All experiments were performed at 37°.

Table 2. Lack of effect of temperature on [^{3}H]nitrendipine uptake*

Temperature (°)	Initial rates (pmoles/mg/min)	Maximal uptake (pmoles/mg)
37	0.0046 ± 0.0002	0.031 ± 0.004
27	0.0049 ± 0.0003	0.032 ± 0.002
17	0.0049 ± 0.0001	0.034 ± 0.001
7	0.0030 ± 0.0003	0.028 ± 0.003

* Nitrendipine uptake into cat ileal smooth muscle was performed in the presence of 10^{-9} M nitrendipine and 1 μCi/ml of [^{3}H]nitrendipine. Calculations of initial rates and maximal uptake were the same as those in Table 1. Data are expressed as mean ± S.E.M. from four experiments.

heart [8–10]. Although we cannot distinguish binding of nitrendipine to the surface membrane and internal membranes, e.g. the SR, it is unlikely that there would be enough binding sites on the surface membrane alone to account for the large T/M ratio. In addition, one would not expect such a long saturation time in the uptake experiments if the binding was only to the sites on the surface membrane.

Other calcium antagonists have also been shown to enter muscle cells. For example, bepridil enters cat ileal smooth muscle, chick embryonic ventricular muscle, rabbit papillary muscle and rabbit aortic rings [4, 11]. Verapamil is accumulated by the myocardium of open-chested dog [12], electrically stimulated atria [13], and in isolated cat ileal smooth muscle and chick ventricular muscle [11].

Although nitrendipine has not been shown to exert internal action in cardiac and smooth muscles, nitrendipine has the potential to do so due to its ability to enter the muscle cells. Other calcium antagonists, e.g. bepridil and verapamil, have been shown to exert internal actions. For example, Mras and Sperelakis [14] have demonstrated that both bepridil and verapamil depress the force of contraction of rabbit aortic rings stimulated to contract in low extracellular Ca^{2+} concentration, i.e. contraction of the smooth muscle is due to internal release of Ca^{2+}. Vogel *et al.* [15] showed that bepridil inhibits the contractions of cardiac muscle more than the slow inward current is depressed, and they suggested that bepridil exerts a second, possibly intracellular, effect, such as an inhibition of Ca^{2+} release from the SR.

The present finding, that cardiac muscle accumulates more nitrendipine than smooth muscle does, could be explained by the more abundant SR and mitochondria in the cardiac muscle. This factor could outweigh the fact that the microsomal fraction from guinea pig ileum can bind more nitrendipine than the microsomal fraction of the rat heart [10].

In summary, nitrendipine entered the smooth muscle cells and cardiac muscle cells and was accumulated, probably through binding to internal sites. Accumulation of nitrendipine was higher in cardiac muscle than in smooth muscle.

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Department of Physiology
University of Virginia School of Medicine
Charlottesville, VA 22908, U.S.A.

DAVID C. PANG*
NICK SPERELAKIS†

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* On sabbatical leave from the Department of Physiology and Biophysics, Medical College of Virginia, Richmond, VA 23298, U.S.A.

† Address all correspondence to: Dr. Nick Sperelakis, Department of Physiology, School of Medicine, University of Virginia, Charlottesville, VA 22908, U.S.A.

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