were significantly higher than for female controls. After 8 h, the values were significantly different for all groups of animals; the highest were observed in males, lower in ovariectomized females and the lowest in control females. After 24 h the OTC plasma levels in all groups of animals decreased below 6 µg/ml, but in males and ovariectomized females they were still significantly higher than in control females. After 48 h, OTC values in the plasma were not measurable. When doses of 10 and 50 mg/kg OTC were injected, the antibiotic could not be detected in plasma 8 and 24 h after the application respectively. It is evident from table 2 that with the dose of 10 mg/kg the concentrations of the antibiotic 1 and 4 h after the application did not vary significantly with the sex of the animal. With the dose of 50 mg/kg, the plasma levels in male and female rats differed significantly only in the later time interval, i.e. 8 h after injection of the antibiotic.

*Discussion.* Studying the influence of sex factors on fatty liver induced by tetracycline, Brenn et al.[7] found no significant differences in the serum levels of antibiotic between male and female rats 3 h after an i.p. application (50–100 mg/kg). However, according to our results, at this early interval sex differences are not likely to be noticeable since they seem to appear at a later stage. The mechanism by which observed differences in plasma levels of OTC occur is uncertain. The metabolic transformation and the volume of distribution seem unlikely to be operative in this case, but the latter possibility cannot be completely excluded in ovariectomized females which have an increased amount of fatty tissue[8]. However, our results indicate that the increased retention of OTC in the bones of male rats observed earlier[4,6] is not necessarily due to differences in the bone turnover but could also be a result of increased OTC level in the plasma. Ovariectomized animals have higher plasma levels than controls. They nearly approach those of male rats indicating that a lack of ovarian sex hormones is partly responsible for the observed effect.

However, it should be kept in mind that the observed sex differences in the plasma levels of OTC might also be of clinical importance, when the antibiotic is administered parenterally in higher doses according to body weight. Further studies are needed to clarify the role of sex factors in OTC metabolism, especially in its elimination, which seems to be of primary importance for explanation of presented data.

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2 Institute for the Control of Drugs, Moše Pijade 160, Y–41000 Zagreb, Yugoslavia.

3 Mailing address: Dr. Dinko Kello.

4 D. A. Buyske, H. M. Eisner and R. G. Kelly, J. Pharmac. exp. Ther. *130*, 150 (1960).

5 K. I. Hawkins, Analyt. Biochem. *45*, 128 (1972).

6 D. Dekanić, PhD Thesis, University of Zagreb, 1975.

7 K. J. Breen, S. Schenker, M. Heimberg, Gastroenterology *69*, 714 (1975).

8 V. Korenchevsky and K. Hall, J. Endocr. *4*, 103 (1945).

## Uptake of noradrenaline in high altitude native's heart

J. R. Rapin, J. Coudert, L. Drouet, J. Durand and Y. Cohen

*Department of Pharmacology and Physiology, University Paris XI, Rue J. B. Clément, F–92290 Chatenay Malabry (France), 22 November 1976*

*Summary.* Uptake of $^3$H-noradrenaline by the heart was studied with sections of isolated atria obtained from high or lowlanders. In native highlanders, affinity for $^3$H-noradrenaline by human atria is more significant than in lowlanders. Furthermore, the Michaelis Menten constant is lower in high altitude native's heart.

People born and residing permanently above 3500 m have a different circulatory pattern from lowlanders. Pulmonary hypertension and right ventricular hypertrophy were described previously[1]. More recently, reduction in regional blood flow and in cardiac output were reported[2–5]. Decrease in local blood supply was accompanied by an increase oxygen arteriovenous difference in such a way that local oxygen consumption was maintained. However, there is an exception: reduction in coronary blood flow was not compensated by a parallel decrease of oxygen content in the blood of the coronary sinus, so that oxygen consumption related to heart weight was reduced in high altitude residents[6].

These results may be related to changes in the noradrenergic nervous system. Studies carried out on rats artifically maintained in conditions of high altitude have demonstrated that the level of cardiac noradrenaline decreased during the period of acclimatization[7–9] and subsequently returned to its normal rate, probably following decreased use of the transmitter. This was parallel to a slight decrease of the turn-over found in hypobaric hypoxia[10], which may be due either to modifications of biosynthesis or to changes in the inactivation of noradrenaline. This paper reports the differences in noradrenaline uptake main route of inactivation of the transmitter, studied on sections of human atria collected at high altitude or low altitude in Andean or European populations.

1 A. Rotta, A. Canepa, A. Hurtado, T. Velasquez and R. Chavez, J. appl. Physiol. *9*, 328 (1956).

2 Y. Bidart, L. Drouet and J. Durand, J. Physiol. *70*, 333 (1975).

3 J. Durand and J. P. Martineaud, Ciba Found. Symposium on High Altitude Physiology 1971, p. 159.

4 J. Durand, J. P. Marc-Vergnes, J. Coudert, M. C. Blayo and J. J. Pocidalo. XXVI int. Congr. Physiol. Sci. Krogh Centenary Symposium, Srinagar, 12–16 October 1974.

5 M. Zelter, J. Mensch-Dechene, G. Antezana, J. Coudert and A. Lockart, IX ann. Meet Eur. Soc. clin. Invest., Rotterdam, 24–26 April 1975.

6 P. R. Moret, Ciba Found. Symposium on High Altitude Physiology 1971, p. 131.

7 J. N. Davis and A. Carlsson, J. Neurochem. *21*, 783 (1973).

8 D. A. Hurwitz, S. M. Robinson and I. Barofsky, Psychopharmacologia (Berl.) *19*, 26 (1971).

9 M. Stupfel, J. Roffi, C. r. Soc. Biol. *155*, 237 (1961).

10 M. Prioux-Guyonneau, J. Durand, J. R. Rapin and Y. Cohen, Experientia *32*, 1024 (1976).

*Material and methods.* 1.7-$^3$H-noradrenaline (7.2 C/mmole) was obtained from the Radiochemical Center Amersham, England, 1-noradrenaline was from Calbiochem. Fragments of right atria were collected during surgery (congenital atrial septal defect) from persons of 20–50 years old, at low or at high altitude. The surgery was performed at high altitude (La Paz, Bolivia, 3,800 m) or at low altitude (Paris, 300 m). In both cases, these patients who had no history of congestive heart failure (i.e. myocardial catecholamines level does not change) were given the same medical treatment prior to surgery. Premedication included curarizing agents or morphine derivatives. After collection, the heart tissue was placed in cooled Mac Ilwain fluid. The 0.5 mm atria sections were obtained by means of a tissue slicer and incubated at 37 °C in 3.5 ml oxygenated Mac Ilwain fluid in the presence of 1-$^3$H-noradrenaline. Incubation was performed in the presence of 1 ng noradrenaline for 2, 5, 10, 15 and 20 min. In another series of tests, the sections were incubated for 10 min in the presence of increasing amounts of noradrenaline (10, 20, 50, 100 and 200 ng per ml of supernatant). After incubation, the sections were rinsed with a cold buffer for a few seconds, dried on filter paper and weighed. They were then homogenized in 0.4 N perchloric acid containing 0.5% EDTA and 0.5% sodium metabisulfite. Noradrenaline was isolated by adsorption on alumina according to Anton and Sayre[11]. Noradrenaline was eluted with 8 ml of 0.4 N $HClO_4$. An aliquot was taken for determination of $^3$H-noradrenaline by liquid scintillation counting. The rest was used for fluorimetric determination of noradrenaline[12]. Recovery of noradrenaline was 79 ± 3%.

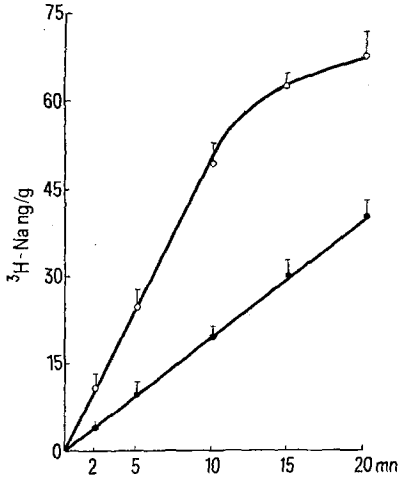


Fig. 1. Time-course of $^3$H-noradrenaline uptake in human atrium sections. The tissue was incubated in 10 ml of Mac Ilwain with 1 ng/ml of $^3$H-NA. ●—● lowlanders atria. ○—○ highlanders atria. Each point represents the mean ± SEM from 5–7 determinations.

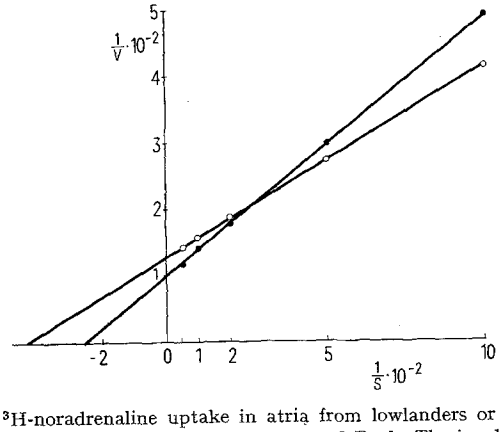


Fig. 2. $^3$H-noradrenaline uptake in atria from lowlanders or highlanders, plotted according to Lineweaver and Burk. The incubation time was 10 min. Each point is the mean of 5 determinations. The line was calculated by linear regression analysis, correlation coefficient = 0.96. Velocity (V) = uptake of $^3$H-noradrenaline ng/g 10 mn. Substrate concentration S = ng/ml $^3$H-noradrenaline.

*Results and discussion.* Figure 1 shows the kinetics of noradrenaline uptake by sections of human atria. Uptake of $^3$H-noradrenaline at a dose of 1 ng/ml was linear up to 20 min when the atrium fragment came from a low altitude resident. In native highlanders, affinity for $^3$H-noradrenaline was more significant than in lowlanders, but there was no linearity after 10 min. Noradrenaline level varied considerably depending on the patient's conditions and treatments. However, the average amounts observed of noradrenaline were 0.95 µg/g ± 0.15 in lowlanders and 1.20 ± 0.20 in native highlanders. Although these variations were not statistically significant, they have an indicative value. Both in lowlanders and in native highlanders, noradrenaline uptake follows Michaelis-Menten kinetics (figure 2). The $K_m$ and $V_{max}$ were determined: (at low altitude, $K_m$ = 0.23 µmoles and $V_{max}$ = 100 ng/g min; at high altitude, $K_m$ = 0.13 and $V_{max}$ = 80).

The results showed that there are differences between affinities for noradrenaline uptake by atria from persons at high or low altitude. Using a 1 ng/ml dose of $^3$H-noradrenaline amine fixation takes place at neurone level and correlates with Iversen's uptake. Neuronal uptake was found to follow Michaelis-Menten kinetics, although the affinity is more significant for the uptake sites of the native highlanders. At low altitude, the values of $K_m$ and $V_{max}$ were of the same order of magnitude as those found in rats using a perfusion technique of the isolated heart according to Iversen et al.[13], and those found in man after incubation of tissue sections[14]. In spite of the in vitro conditions, the endogenous noradrenaline content of the atria was close to the one previously reported[15, 16]. The quantitative difference observed between noradrenaline uptake in lowlanders and highlanders is related to the more significant affinity found at high altitude. This result may be due to a more efficient uptake of noradrenaline by the axon membrane pump, which reduces catecholamine wastage and secondarily induces the slight increase of endogenous noradrenaline. This action should be parallel to the decreased oxygen demand of the heart cells, and the decrease of noradrenaline turn-over rate. However, one may ask what is the triggening factor: is it the function of the membranous pump for noradrenaline, the decrease of the noradrenaline turn-over rate, or the decrease of oxygen consumption?

11 A. H. Anton and D. F. Sayre, J. Pharm. exp. Ther. *138*, 360 (1962).
12 U. S. Euler and F. Lishajko. Acta physiol. scand. *51*, 348 (1961).
13 L. L. Iversen, B. Jarrott, M. A. Simmonds, Br. J. Pharmac. *43*, 845 (1971).
14 C. Sachs and G. Jonsson, Acta pharmac. tox. *31*, 456 (1972).
15 C. A. Chidsey, E. Brauwald, A. G. Morrow and D. T. Mason, New Engl. J. Med. *269*, 653 (1963).
16 L. L. Iversen, The uptake and storage of noradrenaline in sympathetic nerves. University Press, Cambridge 1967.