Zusammenfassung. Chinin (5 m*M*) ruft einen starken Anstieg des Ca^{45} Ausflusses aus den Skelettmuskelfasern von *Carcinus* und *Periplaneta* hervor. Bei dieser Konzentration bewirkt Chinin eine ausgeprägte Hemmung der Kalziumbindung am isolierten sarcoplasmatischen Reticulum und der Mitochondrien dieser Muskeln. Die Hemmung zellulärer Kalziumbindung steht in Wechselbeziehung mit dem Anstieg freien Kalziums und der durch Chinin bewirkten Kontraktionsaktivierung.

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^{3}H-Nortriptyline Uptake and Tissue-Binding in vitro and its Effect on ^{3}H-Noradrenaline Uptake

Tricyclic antidepressants of the nortriptyline (NT) type are known to inhibit the uptake of noradrenaline (NA) into NA nerve terminals both in the peripheral and central nervous system[1–4]. This effect of NT is probably of decisive importance for the antidepressive effect of the drug[5]. NT is a highly lipid-soluble compound and is readily taken up into various tissues and easily passes the blood-brain barrier. With this in mind, it was of interest to study whether NT bound to brain tissue is of importance for the uptake of NA into NA nerve terminals. The present experiments were designed to shed some further light on drug binding to tissue components and to plasma proteins[6]. This binding of drugs is of special interest in in vitro experiments. Clinically it is also important to know if a tissue-bound drug has an inhibitory effect on the uptake of NA.

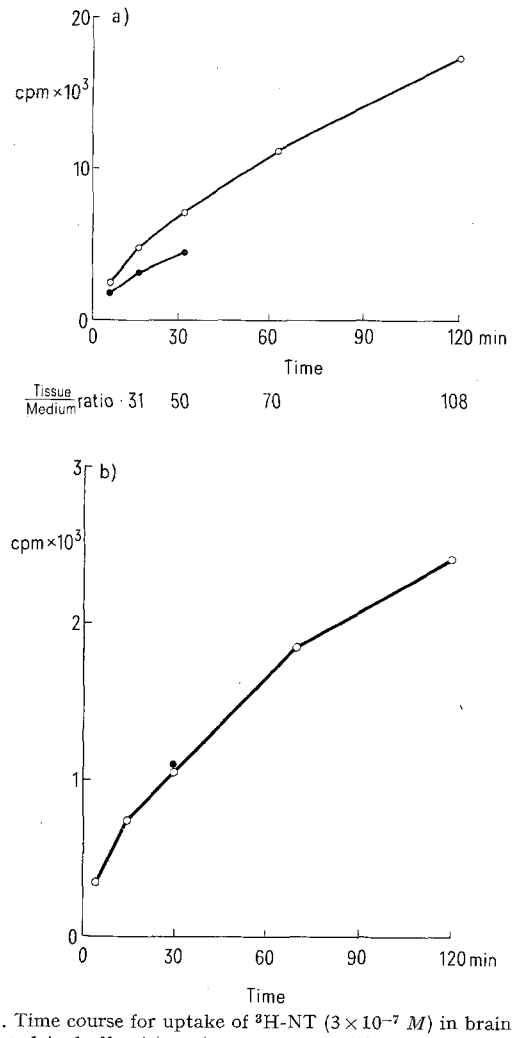

Fig. 1. Time course for uptake of ^{3}H-NT (3×10^{-7} *M*) in brain slices incubated in buffer (a) or human plasma (b). Open circles, 1 mg tissue per ml medium. Closed circles, 10 mg tissue per ml medium. The tissue/medium ratio for uptake in buffer, 1 mg tissue/ml is shown in a). Each value is the mean of 4 determinations.

Methods. Coronal brain slices (0.5 mm thick, 3 mm diameter, weight about 5 mg) from untreated female Spraque-Dawley rats were used. The slices were incubated either in a Krebs-Ringer bicarbonate buffer or in human plasma obtained from the bloodbank. ^{3}H-NA (HCl, 5–10 Ci/m*M*), ^{3}H-NT (HCl, 165 mCi/m*M*), kindly donated by Dr. O. Borgå, and unlabelled nortriptyline (Pharmacia, Sweden) were used in the experiments[4]. The radiochemical purity of ^{3}H-NT was checked with SiO_2 thin layer chromatography (2 *N* ammonia: methanol, 1:4) and was found to be more than 90%.

After the various incubation procedures, the tissue was rapidly rinsed in buffer and the radioactivity determined after solubilization with Soluene® and addition of toluene scintillation solution. Efficiency was determined after addition of ^{3}H-toluene.

Results and discussion. ^{3}H-NT accumulates rapidly in brain slices during incubation in buffer (Figure 1a). After 2 h, a more than 100-fold accumulation of radioactivity has appeared in the slices as compared to the medium. It is reasonable to believe that accumulation of ^{3}H-NT in contrast to ^{3}H-NA accumulation[3] is non-specific and not energy-dependent. The rapid accumulation of NT is most likely related to its high lipid-solubility. When the amount of tissue is increased in the incubation medium from 1 mg to 10 mg tissue/ml medium, the accumulation of ^{3}H-NT in the tissue is less prominent, due to a decrease of the concentration of ^{3}H-NT in the medium (Figure 2). When the incubation is performed in human plasma, ^{3}H-NT accumulation in tissue is almost 10 times less effective (Figure 1b), because more than 90% of ^{3}H-NT is bound to plasma proteins[6]. This implies that both the 'free' concentration of NT in plasma and the accumulation of ^{3}H-NT in slices incubated in plasma are reduced to about 10% compared to the buffer experiments. There seemed to be a fairly rapid equilibration between the 'free' and the plasma protein bound fractions of ^{3}H-NT, since 1 and 10 mg tissue/ml plasma results in the same ^{3}H-NT accumulation in the slices. Thus NT bound to plasma proteins is a reserve pool for the drug.

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The distribution of ^{3}H-NA and ^{3}H-NT between tissue and medium under various incubation procedures is demonstrated in Figure 2. It is evident that in tissue-incubation experiments small amounts of tissue per ml incubation medium should be used in order to obtain exact results. In incubations with ^{3}H-NA only, at the most 10 mg tissue per ml medium should be used, while 80 mg tissue per ml reduces the medium concentration of ^{3}H-NA markedly. With NT in the incubation medium, both 10 mg and especially 80 mg tissue per ml produces a marked reduction of the NT concentration in the medium. From these experiments it is apparent that with highly lipid-soluble drugs like NT, only 1 mg tissue per ml ought to be used in incubation experiments. Variations in tissue-amount per ml medium and incubation-time cause at least a 3-fold variation in the NA uptake inhibition by NT[7]. It is of great importance to keep the medium concentration of NT constant when studying its effect on the uptake of NA. In the Table this has been done by having sufficient medium per slice. The preincubation time with NT was varied in order to obtain varying tissue concentrations of NT and the uptake of ^{3}H-NA determined. There are only small variations in the inhibition of ^{3}H-NA uptake both in buffer and plasma, in spite of large variations in tissue NT concentrations (cf. Figure 1). This finding is further illustrated in Figure 3, demonstrating that the tissue-bound NT is of minor if any importance for ^{3}H-NA uptake inhibition[8].

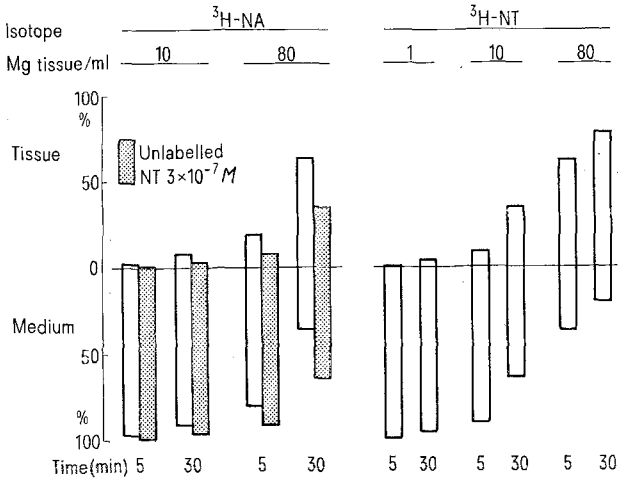

Fig. 2. Distribution of ^{3}H-NA and ^{3}H-NT between tissue and medium after incubation of brain slices in Krebs-Ringer bicarbonate buffer. The values are expressed as % of total radioactivity (dpm) present in tissue and medium after the incubation. ^{3}H-NA concentration 2×10^{-8} M, ^{3}H-NT concentration 3×10^{-7} M. Closed bars illustrate ^{3}H-NA experiments where unlabelled NT was present 15 min before and during incubation with ^{3}H-NA. The amount of tissue per mel incubation medium and the incubation period with isotope was varied as shown. Each value is the mean of 4 determinations.

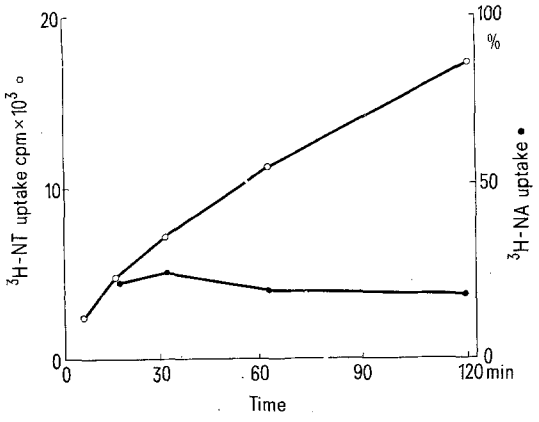

Fig. 3. ^{3}H-NT uptake and ^{3}H-NA uptake (% of NT-free control) in brain slices incubated in buffer. NT concentration 3×10^{-7} M. The values are from the Figure and the Table.

Effect of various preincubation periods with nortriptyline (NT) on ^{3}H-noradrenaline (^{3}H-NA) uptakte in brain slices

Medium	Preincubation time (min)	Concentration of NT in medium (M)	Uptake of ^{3}H-NA % of NT-free control
Buffer	15	3×10^{-8}	43 ± 2
	30		45 ± 1
	2×30		34 ± 1
	4×30		33 ± 1
Buffer	15	3×10^{-7}	23 ± 1
	30		25 ± 1
	2×30		20 ± 2
	4×30		18 ± 1
Plasma	15	3×10^{-7}	38 ± 2
	30		35 ± 2
	2×30		36 ± 1

The tissue amount was 1 mg/ml buffer and 10 mg/ml plasma. Incubation time with ^{3}H-NA 5 min. The marked increases in NT concentrations in the tissues can be seen in figure 1. Mean $\pm$ s.e.m. of 4 slices.

In chronic administration of NT, steady-state kinetics are obtained after approximately 6 days[8]. It is also known from clinical experience that there is a delay between treatment's initiation and the first signs of amelioration. This might be a result of the binding of the drug to tissue. Thus, until the tissue has been saturated, there may not be sufficient 'free' NT available to cause an uptake inhibition in the central NA nerve terminals, although brain content of bound NT may be relatively high[9].

Zusammenfassung. Inkubierung von Gehirnschnitten in Puffer mit ^{3}H-Nortriptylin gibt eine mehr als 100fache Ansammlung von ^{3}H-Nortriptylin im Gewebe. Wenn die Inkubierung im Plasma geschieht, ist die Ansammlung von ^{3}H-Nortriptylin abhängig von der Konzentration der «freien» Droge im Plasma. Diese «freie» Konzentration verursacht die Hemmung der Noradrenalinaufnahme in Nerventerminalen der Hirnrinde und das gewebegebundene Nortriptylin ist, wenn überhaupt, von geringer Bedeutung für die Hemmung der Noradrenalinaufnahme.

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