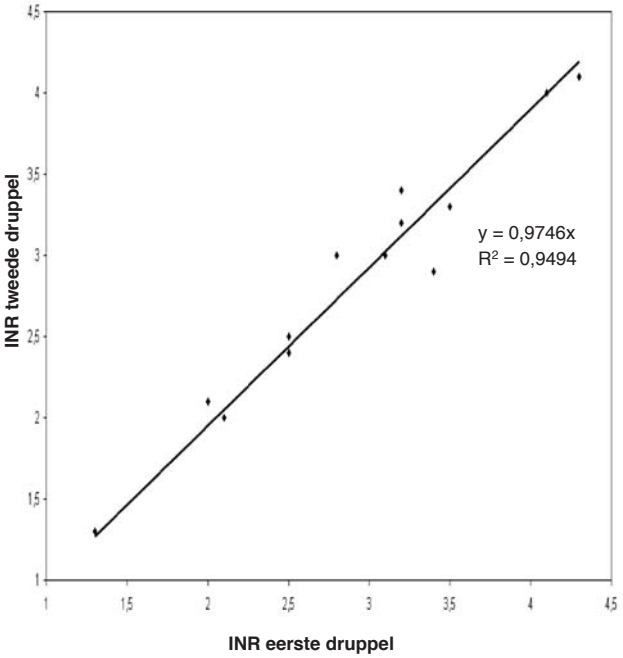

Figuur 1. De correlatie tussen de INR-uitslagen gemeten met de eerste druppel en met de tweede druppel capillair bloed.

iedere meting en hetzelfde apparaat. Van de uitslagen is de lineaire regressie bepaald en de correlatie (Pearson). Bij alle patiënten wordt ook standaard een veneuze INR bepaald op een ACL Futura met Recombiplastin.

Resultaat
De uitslagen van de eerste en de tweede INR-bepaling hebben een goede correlatie met een hoge (0,97) r-waarde. Wel werd waargenomen dat dit alleen geldt als beide bepalingen kort na elkaar worden gedaan, de INR fluctueert gedurende de dag; sommige patiënten hadden al eerder op de dag thuis een INR bepaald. Het verschil tussen de capillaire INR en de veneuze INR was dan soms groter dan 0,5 INR. De afwijking ten opzichte van de venapunctie is soms groter dan 0,5 INR.

Conclusie
Er is geen statistisch relevant verschil tussen de INR-bepaling op de CoaguChek S van Roche tussen de primaire druppel en de secundaire druppel na afvegen bij ervaren patiënten, mits het tijdsverschil tussen de beide bepalingen kort is. Het verschil neemt toe zodra de tijdsinterval tussen de twee metingen toeneemt. Dit laatste kan verklaard worden door het feit dat patiënten vaak acenocoumarol gebruiken, een geneesmiddel met een halfwaardetijd van ongeveer 12 uur, hetgeen bij dezelfde patiënt binnen een dag reeds INR-schommelingen laat zien. Daarom wordt bij zelfmetende patiënten geadviseerd om wekelijks op dezelfde dag en op het zelfde tijdstip te meten. De observatie tussen het verschil in INR-uitslagen tussen capillaire en veneuze INR wordt nader uitgezocht. Momenteel wordt door de Federatie van Nederlandse Trombosediensten geadviseerd om bij verschillen groter dan 0,5 INR de patiënt te ontraden om de CoaguChek te gebruiken.

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Norepinephrine detection in "Tree Shrews" urine using LC-Tandem MS to verify that stress-induced alterations are prevented by the NK1 receptor antagonist SLV 323

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Introduction

Pharmacological
Substance P and its receptor, the neurokinin 1 (NK1R) have been discussed as possible targets for new antidepressant therapies (1). Here we investigated the therapeutical potentials of the NK1R antagonist SLV 323 in the chronic psychosocial stress paradigm of adult male tree shrews (2). Animals were subjected to a 7-day period of stress before the onset of daily oral administration of SLV 323 (20 mg/kg/day). The stress continued throughout the treatment period of 28 days. Urine samples were collected daily for determination (among others) of noradrenaline and creatinine.

Analytical
Analytical methods were developed and "in study" validated. The Jaffe's colorimetric creatinine analysis method is considered as a well known assay and not described. The isolation [3] of catecholamines with liquid liquid based extraction using complex formation in combination with ion pairing is more or less unknown probably due to the relative complexicity of the composition of reagents and/or extraction proce-

KCL BioAnalysis[1], Leeuwarden; Solvay Pharmaceuticals[2], Weesp, The Netherlands; Clinical Neurobiology Laboratory[3], German Primate Center, Goettingen, Germany

Table 1. Analytical evaluation of the method

Absolute recovery based on peak area; assay peak/mobile phase peak	
Norepinephrine	96,3 % at 165 nmol/l, n=6
Dihydroxybenzylamine (IS)	91,7 % at 500 nmol/l, n=5
Functional assay range	78 to 10000 nmol/l Norepinephrine
Suitability control in nmol/l	201 NEN and 2000 DHBA in mobile phase
Suitability within run precision	CV % = 2.6, DF = 82
Calculation model	y = a*x + b; using weight; 1/x
Back calculated values	Mean bias: -1.1 %, DF = 90

Quality control	nmol/l	Interassay CV (%)	Mean Bias (%)	DF
QC Low	177	9.7	1.1	23
QC Medium	827	5.6	-5.8	22
QC High	4016	3.8	-5.4	23

dure. In this study urine samples were extracted and measured using the LC-MS/MS (API 3000). Established key figures are presenting the analytical method. The analytical part of the study was conducted in accordance with GLP.

Methods

Stress was provoked by putting together a dominant and a subdominant tree schrew in a cage for 1 hour. The rest of the day both animals were put into different cages that were placed adjacent so the animals could both see and smell eachother. Four cohorts were created: control (no stress), treated control (no stress, treated with SLV 323), stress (no treatment), stress with treatment (stressed animals, treated with SLV 323).

Urine from the tree shrews was collected daily by gently rubbing their abdomens and was frozen in plain cups for creatinine analysis and for catecholamine analysis in cups to which 30 µl 6 N HCL per ml urine was added. Prior to chromatographic analysis, catecholamines were extracted from the urine samples:

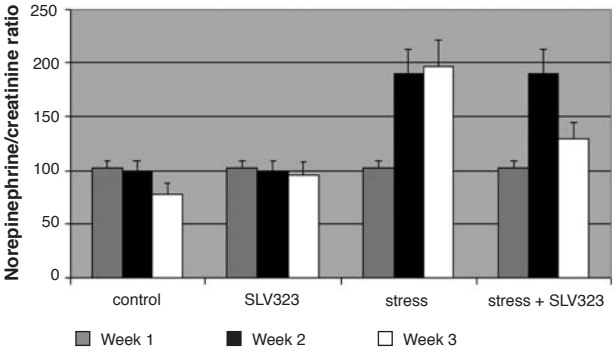

Figure 1. Effect of chronic psychosocial stress and treatment with an NK1R antagonist on urinary norepinephrine.

Catecholamines specifically reacted in the water phase with diphenylborate (in the presence of EDTA) forming a negatively charged complex at pH=8,95 with $[NH_4]^+$ as paring ion. The complex migrated into a heptane/octanol organic phase containing tetraoctylammoniumbromide as counterion for the complex. Following extraction,the catecolamines were set free by acid hydrolysis (acetic acid) and migrated back into a water phase. The catecholamines were analysed in the waterphase by LC tandem MS. The LC tandem MS analysis was optimised for norepinephrine but could also detect dopamine and epinephrine in the same run.

Results

The results are given in table 1 and in figure 1.

Conclusions

Chronic psychosocial stress induced an increase in urine-levels of norepinephrine. These stress effects were prevented by concomitant administration of the NK1R antagonist yielding normal values and thus suggesting that SLV 323 is able to counteract stress-induced neuroendocrine alterations.

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