Effect of serum on adrenal PNMT activity

Short Communication

N. Matussek, L. Deuringer, and I. Sardas-Trevorrow

Department of Neurochemistry, Psychiatric Hospital, University of Munich, Federal Republic of Germany

Accepted April 21, 1992

Summary. Humoral mechanism should be responsible for activation of PNMT (phenylethanolamine N-methyltransferase) by CRF (corticotropin-releasing factor) in vivo (Lima and Sourkes, 1987). Small amounts of serum (10 μl) caused a sig. dose dependent activation of bovine PNMT activity (19.0 ± 1.8%) changing in $K_m$ and $V_{max}$ values in vitro. Human/rat or ovine CRF – in high amounts (8.4 nmol) only – increase at the rate of just 10% bovine PNMT activity in vitro. At the moment, we do not know which factor in serum is responsible for the increase in A (adrenalin) synthesis in vitro.

Keywords: PNMT-activation-serum.

Introduction

Lima and Sourkes (1987) showed an increased activity of adrenal dopamine beta-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT) by intraventricular administration of corticotropin-releasing factor (CRF). Because “denervation of the adrenal gland limited the elevation of DBH activity, but did not significantly affect the PNMT activity”, they concluded “that a humoral mechanism functions in the case of PNMT activation”. During our studies to determine PNMT activity in the blood of depressed patients, we observed in vitro an activation of bovine PNMT by human serum.

Material and methods

L-noradrenaline (NA) bitartrate, L-adrenalin (A) bitartrate, charcoal (14–60 mesh), human/rat and ovine corticotropin-releasing factor (CRF) were obtained from Sigma-Chemie, bovine PNMT from Boehringer Mannheim, 3, 4-dihydroxybenzylamin (DHBA) from RBJ
and SKF 64139 A from Smith, Kline and French. HPLC column: C_{18} Spherisorb ODS II 125 mm x 4.0 mm Phase Separations Fa. Bischoff, Germany.

Determination of PNMT activity was accomplished with 1.1 U PNMT according to the methods of Trocewicz et al. (1982)

Blood from three healthy males and two healthy females (age 35–69 y) was obtained in prone position 20 min after inserting a cannula in a brachial vene, in cold monovettes. After 10 min on ice, the blood was centrifuged at 4 °C for 20 min at 1200 g. Serum was then divided into 1 ml samples and stored at −80 °C.

**Results and discussion**

Addition of a small volume of serum (10 µl) to bovine PNMT caused a sig. dose dependent increase in the adrenalin (= A) synthesis of 19.0% ± 1.8 (Fig. 1). Higher amounts of serum, as 50 µl and 100 µl, did not stimulate the A synthesis beyond the level reached with 10 µl. Our small group of probands did not show gender differences in the stimulation of A synthesis with serum.

K_{m} and V_{max} values of PNMT are changed through addition of serum (Table 1).

The K_{m} value is nearly doubled and the V_{max} value increases by 33% through addition of serum.

SKF 64139 A (1 × 10^{-5} mol), a quite specific PNMT inhibitor, totally blocked A-synthesis, also in combination with serum. When serum is heated up to 50 °C or 100 °C for 10 min, the A forming activity of PNMT is being reduced ~ 20% or 80%, respectively. We do not know whether the activating property is changed into inhibiting property by heating of serum, or whether it is a destruction of the activating property.

Five % of charcoal-treated serum (Arriza et al., 1987) show the same activating effect as untreated serum.

PNMT activity or other non-specific N-methyltransferases in serum cannot

![Graph](image)

**Fig. 1.** Activation in percent of bovine PNMT (1.1 U) by different amounts of serum (n = 6). For experimental details see Material and methods. Data are expressed as mean and sd