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EFFECTS OF N-(2-PROPYLPENTANOYL)UREA ON NMDA RECEPTOR SUBTYPE NR1A/NR2B EXPRESSED IN *XENOPUS LAEVIS* OOCYTES

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วัตถุประสงค์หลักของการศึกษาในครั้งนี้ คือการหากลไกในการต้านชักของสารเอ็น-(2-โพรพิลเพนทาโนอิล)ยูเรีย หรือวีพียู ซึ่งเป็นอนุพันธ์ใหม่ของวาลโปรเอทที่สังเคราะห์ขึ้นมา โดยจะศึกษาผลของสารดังกล่าวต่อตัวรับเอ็นเอ็มดีเอ ชนิดเอ็นอาร์เอ1เอ/เอ็นอาร์2บี ซึ่งถูกเหนี่ยวนำให้แสดงออกบนผิวเซลล์ไข่กบสายพันธุ์*ซีโนปุส* ด้วยการฉีดซีอาร์เอ็นเอ และใช้เทคนิคการวัดกระแสที่ศักย์ไฟฟ้าคงที่ด้วยขั้วไฟฟ้า 2 ขั้ว

สารวีพียูในขนาด 1-300 ไมโครโมลาร์ ไม่มีผลเปลี่ยนแปลงต่อการนำไฟฟ้าของไข่กบ สายพันธุ์*ซีโนปุส* แต่การให้กลูตาเมทขนาด 0.01-300 ไมโครโมลาร์ ร่วมกับกลัยซีนในขนาด 10 ไมโครโมลาร์ จะทำให้เกิดกระแสไหลผ่านเข้าสู่เซลล์ไข่กบในปริมาณที่แปรตามความเข้มข้นของกลูตาเมทที่ให้ โดยมีค่าความเข้มข้นที่จะทำให้เกิดการตอบสนองกึ่งหนึ่งของการตอบสนองสูงสุดที่ 2.26 ± 0.31 ไมโครโมลาร์ วีพียูในขนาด 100-300 ไมโครโมลาร์ สามารถยับยั้งฤทธิ์ของกลูตาเมทที่มีต่อตัวรับเอ็นเอ็มดีเอ ชนิดเอ็นอาร์เอ1เอ/เอ็นอาร์2บี ได้ในเชิงแข่งขันและผันกลับได้ ดังจะเห็นจากการเลื่อนกราฟแสดงการตอบสนองของกลูตาเมทออกไปทางด้านขวาโดยไม่เปลี่ยนแปลงระดับการตอบสนองสูงสุด

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PENPHIMON PONSUP : EFFECTS OF N-(2-PROPYLPENTANOYL)UREA ON NMDA RECEPTOR SUBTYPE NR1A/NR2B EXPRESSED IN *XENOPUS LAEVIS* OOCYTES . THESIS ADVISOR : ASSOC.PROF. BOONYONG TANTISIRA, Ph.D., THESIS COADVISOR : ASST.PROF. PATHAMA LEEWANICH, Ph.D., 91 pp. ISBN 974-17-3917-6.

The main goal of this study was to gain insight into the mechanisms underlying the anticonvulsant activity of N-(2-Propylpentanoyl)Urea (VPU), a new synthetic valproate derivative, on NR1A/NR2B NMDA receptors subtype using the two-electrode voltage-clamp technique in *Xenopus laevis* oocytes injected with cRNAs.

Application of VPU (1-300 μM) produced no changes on the membrane conductance of *Xenopus* oocytes. However, coapplication of glutamate (0.01 - 300 μM) and 10μM glycine induced inward currents with a dose-response curve giving the EC_{50} of glutamate at 2.26±0.31 μM. Whereas 1 mM of VPA produced very marginal effect, VPU (100- 300 μM) exerted a reversible and competitive inhibition of glutamate response on NR1A/NR2B NMDA receptor characterized by shifting the glutamate concentration curve to the right with no alteration of maximal response. Similar response was elicited by AP5, a selective, competitive NMDA binding site antagonist at the concentration of 10 μM. Thus the inhibitory of VPU seems to be comparatively weaker than AP5. Furthermore, based on the results that the inhibitory effects of 100 μM VPU on inward currents induced by 3 μM glutamate did not alter either when the holding potential was stepwise increased from -150 to + 50mV or when different concentration of glycine, a co-agonist of glutamate, was co-applied, it is apparent that antagonistic effect of VPU on glutamate-induced inward current was neither voltage nor glycine dependent. In addition, it was found that VPU (100-300 μM) significantly decrease stimulatory effect of spermine on glutamate response. Taken into consideration that polyamines has been reported to be increased on the face of seizure or ischemia, it is suggestive that inhibitory effect of VPU on NR1A/NR2B NMDA receptors, though rather weak, may, in concert with its effect on $GABA_A$ receptor and perhaps with some other mechanism that remain to be identified, contribute to its anticonvulsant effect in vivo.

Department	Physiology	Student's signature	Penphimon Ponsup
Field of study	Physiology	Advisor's signature	Boonyong Tantisira
Academic year	2003	Co-advisor's signature	Pathama Leewanich

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LIST OF ABBREVIATIONS

μ	=	Micro
μM	=	Micromolar
%	=	Percent
K^+	=	Potassium ion
Ca^{2+}	=	calcium ion
Mg^{2+}	=	Magnesium ion
K^+	=	Potassium ion
Cl^-	=	Chloride ion
Na^+	=	Sodium ion
Zn^{2+}	=	Zinc ion
AEDs	=	Antiepileptic drugs
AMPA	=	α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid
CNS	=	Central Nervous System
DNA	=	Deoxyribonucleic acid
ED_{50}	=	Median effective dose
et al.	=	et alii (and other)
GABA	=	Gamma aminobutyric acid
GluRs	=	Glutamate receptors
IC_{50}	=	Median inhibitory concentration
M	=	Molar
MΩ	=	Megaohm
MBS	=	Modified Barth's Solution
mg	=	Milligram
ml	=	Milliliter
nl	=	Nanoliter
ng	=	Nanogram
NMDA	=	N-methyl-D-aspartate
S.E.M.	=	Standard error of the mean