J. Pharm. Pharmacol. 1998, 50 (Supplement): 213

Effect of membrane cholesterol enrichment on the potentiation of $GABA_A$ currents by neurosteroids

THONGCHAI SOOKSAWATE AND MICHAEL A. SIMMONDS

Department of Pharmacology, The School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX

There is much evidence that cholesterol acts as a direct modulator of many membrane proteins but the mechanisms involved are still unclear (Yeagle, 1991). An initial study on the $GABA_A$ receptor in neuronal membrane fragments has shown that enhancement of ^{3}H-flunitrazepam binding by other classes of GABA-potentiating drugs is affected by cholesterol enrichment of the membranes (Bennett and Simmonds, 1996). We now report the effects of cholesterol enrichment of the membrane of whole neurones on the potentiation of $GABA_A$ currents by neurosteroids.

Brain slices containing hippocampus from 10-16 days old male Wistar rats were incubated in 0.03% pronase for 20 min at 31°C followed by 0.03% thermolysin for 20 min at 31°C in physiological salt solution (PSS) containing (mM): NaCl 140, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, glucose 11, HEPES 10 (pH 7.4). The hippocampal neurones were dissociated by trituration with glass pipettes. The tissue remaining suspended after standing for 10 min was centrifuged at 175 xg for 4 min to form a very loose pellet which was then layered onto a 5% solution of bovine serum albumin (BSA) in PSS for further centrifugation to separate a loose pellet of neurones from suspended cell debris. Enrichment of neurones with cholesterol was achieved by incubation at 31°C for 60 min in oxygen-saturated PSS containing 1% BSA and cholesterol+phosphatidylcholine liposomes at a final cholesterol concentration of 0.15-0.20 mg.ml^{-1}. Unenriched neurones were subjected to the same processes but without liposomes. Electrophysiological recordings were made from dissociated single neurones which adhered to the bottom of the recording chamber. Whole cell membrane currents were recorded with patch pipettes of 3-5 MΩ resistance at room temperature (~20°C) and the membrane voltage clamped at -20 mV. The patch pipette solution contained (mM): CsCl 140, $CaCl_2$ 1, $MgCl_2$ 2, Na_2ATP 2, EGTA 11, HEPES 10 (pH 7.2). Neurosteroids were dissolved in dimethylsulfoxide (DMSO) at a concentration of 10 mM and diluted to 1 μM in PSS. The concentration of DMSO was less than 0.05% throughout. Drug was applied close to the recorded neurone by the "U-tube" adaptation from the "Y-tube" method (Wakamori *et. al.*, 1993). Full log. concentration-response curves were constructed over the range 0.1-300 μM GABA.

The cholesterol enrichment procedure yielded a neuronal cholesterol of 0.59±0.03 μmoles.mg protein^{-1} compared with the value of 0.47±0.02 μmoles.mg protein^{-1}, (P<0.005, Student's unpaired t-test, n=17) in unenriched neurones. The percentage enrichment was 25.8±3.4%. Results from the analysis of the log.concentration-response curves are shown below. Cholesterol enrichment had little effect on the potency of GABA but it decreased the potentiation of GABA by each of the neurosteroids tested.

GABA+Drug	Cholesterol level	Log EC_{50}
GABA alone	Unenriched (n=8)	-5.306±0.017
	Enriched (n=9)	-5.262±0.020
Pregnanolone	Unenriched (n=8)	-6.086±0.089
	Enriched (n=9)	-5.732±0.071*
Allopregnanolone	Unenriched (n=12)	-6.367±0.078
	Enriched (n=12)	-5.912±0.067*
Alphaxalone	Unenriched (n=6)	-5.851±0.020
	Enriched (n=9)	-5.629±0.041*

*different from unenriched neurones (P<0.005, unpaired t-test)

These results suggest that cholesterol within the neuronal membrane may compete with neurosteroids for their sites of action on the $GABA_A$ receptor or selectively modulate the potentiating effect of the neurosteroids in some other ways.

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