TITLE: METHYLGUANIDINE INCREASES EVOKED RELEASE OF ACETYLCHOLINE FROM RAT BRAIN

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It has been reported that methylguanidine (MG) accumulates in the body of uremic patients. While MG serum concentration in normal subjects was < 4 μM, it was 44.4±5.7 μM (Mean±SEM) in these patients.[1] It was also reported that guanidine and its derivatives increase the acetylcholine (ACh) release at the neuromuscular junction.[2] In this study, effects of MG on ACh release from rat brain were investigated.

Rat brain cortical or hippocampal slices, loaded with [^{3}H] choline, were electrically stimulated 2 times (S1 and S2), 40 min apart for 2 min at 1 Hz, while continuously superfused with 0.4 ml/min Krebs' solution containing 10 μM hemicholinium-3. Except for control experiment, MG was added between S1 and S2 to the superfusing solution. Five min (2 ml) aliquots of the superfusate were collected with a fraction collector, and the radioactivity of the samples were measured by scintillation spectrometry. The stimulation evoked release of [^{3}H] ACh was calculated by subtracting the basal release of [^{3}H] from the total [^{3}H] release. Increase of the ratios of evoked release by S2 and S1 (S2/S1 ratio) by the addition of a drug indicates facilitation, its decrease inhibition of ACh release.

In the absence of drugs, the S2/S1 ratios were constant in both cortex and hippocampus. MG dose-dependently increased the S2/S1 ratio both in cortex and hippocampus (Table, Mean±SEM of 4-7 experiments).

MG increases ACh release from rat brain cortex and hippocampus in concentrations which have been found in sera of patients in renal failure. The increased ACh release caused by this compound may contribute to some of the CNS manifestations (e.g. convulsions) encountered in renal failure, and may cause abnormal responses to anesthetic drugs in these patients.

REFERENCES.
1. Clin Chim Acta 82:141-150, 1978
2. Neurosci 4:1511-1519, 1979

Table

Conc. of MG (μM)	S2/S1 Ratio		% Changes of S2/S1	
	Cortex	Hippocampus	Cortex	Hippocampus
0 (Control)	0.78±0.03*	1.02±0.09	-	-
30	1.02±0.08**	1.37±0.07	32±10	34± 7
100	1.39±0.06**	1.75±0.16**	79± 8	71±15
300	1.48±0.11**	2.40±0.26**	91±14	135±26
1000	1.67±0.05	3.43±0.31	116± 7	236±31

* and ** indicate significant differences from control at $p < 0.05$ and $p < 0.01$, respectively (ANOVA followed by Dunnett's test).

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Title: Thiopental Attenuates Anoxia - Induced Na and K Concentration Changes in the Rat Hippocampal Slice

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Thiopental provides protection against anoxia in the in-vitro rat hippocampal slice model (1) and improves outcome following focal cerebral ischemia. A variety of protective mechanisms have been invoked for thiopental. Blocking Na influx during anoxia enhances neuronal resistance to oxygen deprivation (2). We therefore examined the effect of 600 uM thiopental on anoxic Na and K levels in the rat hippocampal slice preparation.

Methods: The slices were prepared as previously described (1) and were immersed for 2 hrs. in oxygenated artificial CSF. When used, thiopental was added to aCSF 15 mins. before anoxia, which was generated by substituting N_2 for O_2 for 10 mins. The slices were removed from aCSF at the end of anoxia and immersed for 10 mins. in an ice-cold isoosmotic sucrose solution to rinse out the excess aCSF. The slices were then dried in an oven for 48 hrs. After dry weights were determined, the slices were digested with O.1 N HNO_3 and Na and K assayed using a flame photometer. The Na and K levels obtained reflect the intracellular and extracellular electrolyte content of the slices. Statistical significance was determined using ANOVA and t-test.

Results: Na levels increase by 55% during anoxia when no drug is present. The actual ion levels are shown in the table. 600 uM thiopental - treated slices' Na content increases only by 21% during anoxia. Therefore, thiopental significantly reduces the anoxic rise in Na levels in the slices. K concentration in the whole slice decreases to 48% during anoxia in the absence of thiopental and to 69% in its presence. Thus thiopental significantly attenuates the anoxic K level decrease in the slices.

Conclusion: It was found that 600 uM thiopental attenuates the slice Na concentration increase and K decrease due to anoxia. This is probably due to a decrease in Na influx and K efflux. Increased intracellular Na may cause an increase in cytoplasmic Ca levels by opening voltage - dependent cell membrane Ca channels or by mitochondrial release of Ca. Ca is believed to trigger the autolytic processes ultimately leading to cellular death from anoxia. Increased extracellular K may enhance these changes causing further depolarization through the spreading depression mechanism. We postulate that thiopental's protective properties might be in part due to its ability to either delay or decrease the amplitude of anoxic depolarization.

Table: Na and K concentration in the slice (mM/mg dry wt.) (x+SEM)

	(A) No Drug Normoxia	(B) No drug Anoxia	(C) Thiopental 600uM Anoxia
Na	2.51+0.16	3.90+0.07*	3.04+0.07**
K	1.86+0.09	0.90+0.04*	1.29+0.03**

***p<0.001 BvsA **p<0.001 CvsB**

References: (1) Brain Research, 403 (1987) 136-141
(2) Neuroscience, 33 (1989) 263-268