NeuroReport 10, 3049–3053 (1999)

# Uridine is released by depolarization and inhibits unit activity in the rat hippocampus

**Á. Dobolyi, T. Szikra, A. K. Kékesi, Zs. Kovács and G. Juhász[CA]**

Department of Physiology and Neurobiology, Eötvös Loránd University, H-1088 Budapest, Múzeum krt. 4/a, Hungary

[CA]Corresponding Author

**PERFUSION of 5 μM kainate through microdialysis probes induced > 2-fold elevation of extracellular uridine and adenosine concentrations in the hippocampus and in the thalamus of anaesthetized rats. Administration of uridine via this route produced an estimated uridine concentration of 50–100 μM around the electrode surface. This markedly decreased the average firing rate of neurones in the hippocampus, but not in the thalamus. Activity of separated single hippocampal pyramidal cells was completely inhibited by uridine. The same amount of adenosine completely blocked neuronal activity in both hippocampus and thalamus. Uridine administration had no effect on extracellular adenosine concentration. These findings suggest an important neuromodulatory role for depolarization-released uridine in the CNS. *NeuroReport* 10:3049–3053**


**Key words**: Adenosine; Extracellular unit activity; Hippocampus; *In vivo* release; Neuromodulator; Uridine

## Introduction

Nucleotides and nucleosides are signalling molecule candidates in the nervous system since there is an increasing amount of evidence about their neuromodulatory effects *in vitro*. Adenosine is a neuroprotective agent [1] and it also has neuromodulatory function [2]. Adenosine can be formed from the established neurotransmitter and neuromodulator [3] ATP in the extracellular space [4], and stimulus-induced release of adenosine was also described [5–7]. The recent cloning of different types of pyrimidine receptors [8] and the various effects of UTP and UDP on the intracellular $Ca^{2+}$ levels and membrane currents of brain cells [9–11] suggest that UTP and UDP are putative signalling molecules [9,12]. Extracellular ATP degrading enzymes convert UTP to uridine [4]. Plasma concentration of uridine shows circadian rhythm [14] and it affects sleep in rats [13,15]. Behavioural studies suggest an interaction of uridine with the striatal dopaminerg system [16]. Concerning cellular mechanisms, the recently reported uridine-activated fast transmembrane $Ca^{2+}$ ion fluxes [17] raised the need for studies on effects of uridine on neuronal activity.

In the present study, microdialysis probes were implanted into the hippocampus and ventro-posterolateral/ventro-posteromedial (VPL/VPM) thalamic nuclei where inhibition-induced calcium spike-based synchronized activities were obtained in relation to the sleep–waking cycle [18,19]. Uridine and adenosine releases were investigated in response to chemical depolarization. Additionally, the effect of uridine and adenosine application through microdialysis probes on unit activity was measured with tungsten microelectrodes attached 400 μm apart to the microdialysis probes. To estimate the extracellular concentration of the applied nucleosides at the recording electrodes, double microdialysis probe experiments were performed. To exclude the possibility that uridine inhibits neuronal firing rates due to elevation of adenosine concentrations via linked metabolic pathways, the effect of uridine on the extracellular concentration of adenosine was also tested.

## Materials and Methods

*Materials:* Kainate, TTX, uridine and adenosine were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA; ≥ 99% purity). HPLC grade organic solvents and HPLC eluent components were products of Merck Co. (Darmstadt, Germany).

*Surgery:* Experiments were carried out on the basis of local ethical rules in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines, the European Communities Council Directive 24 November 1986 (86/609/EEC). Under anaesthesia in a halothane–air mixture (0.5–0.8% halothane–air mixture, 1400 ml/min flow rate), microdialysis probes and electrodes were implanted into 350–400 g rats.

*Microdialysis:* Microdialysis probes were made as

described previously [20]. Briefly, hollow fibers (Travenol, cut-off at 5000 da, od. 0.2 mm, length of the active surface 1.5 mm) were adjusted into 25-Gauge stainless steel tubings. Glass capillaries pulled from Jencons glass and guided by stainless steel tubes of 27-Gauge were used as inlet and outlet of the probe. In double probe experiments, two microdialysis probes were attached together at a distance of 400 μm.

Microdialysis probes were placed to the following coordinates on the basis of the rat brain atlas of Paxinos and Watson [21]: thalamus: A −3.1, L 2.7, V −6.7 mm; hippocampus: A −3.1, L 2.7, V −3.9 mm. In double probe experiments the uridine-injecting probe was placed at A −3.1, L 2.1, V −3.9 mm, and the test probe was at A −3.1, L 2.7, V −3.9 mm.

Probes were inserted slowly, in not less than 20 min, in order to reduce tissue damage. Artificial cerebrospinal fluid (ACSF) containing 144 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$ and 2 mM $CaCl_2$, pH = 7.0 was used for perfusion. The flow rate was 2 μl/min. A 1 h recovery period was allowed before starting sample collection. Two control samples of 60 μl were collected, then the perfusion fluid was changed to ACSF containing kainate (5 μM), kainate (5 μM) plus TTX (1 μM) or uridine (10 mM). Two samples were obtained under drug perfusion then the perfusion fluid was changed back to ACSF to collect two recovery samples. In the double probe experiments, ACSF was changed to uridine-containing ACSF in the injection probe and the test probe was continuously perfused with ACSF. At the end of the experiments, rats were overdosed with 4% halothane–air mixture and coronal sections were cut for Nissl-staining.

*HPLC analysis of uridine and adenosine:* Samples of 60 μl were collected every 30 min and 50 μl of each were used for nucleoside assay. Our recently developed, extremely sensitive and selective HPLC method was used to measure uridine and adenosine simultaneously in microdialysis samples [22]. Briefly, separation was performed on a Pharmacia LKB SMART system using a Hewlett-Packard Hypersil ODS reversed-phase, C18 column (200 × 2.1 mm). Eluent A was 0.02 M formiate buffer containing 0.55% acetonitrile, pH 4.45. Eluent B was 0.02 M formiate buffer containing 40% acetonitrile, pH 4.45. The gradient profile was as follows: 0% B eluent at 0–10 min, 10% at 22 min, and 100% at 30 min. The flow rate was 350 μl/min. The column temperature was 10°C. Compounds were detected by u.v. at 254 nm. The detection limit was 0.5 pmol for uridine and 0.1 pmol for adenosine in samples of 50 μl.

*Measuring unit activity in combination with microdialysis:* Electrodes were made from tungsten wires (20 μm diameter), edged in potassium nitrite solution with AC current and insulated with epoxy varnish (Clark EPR-4). Electrode tips were scraped under a microscope. Electrodes were attached parallel to the microdialysis probes 400 μm from the probe wall. Vertically, the electrode tip was at the middle of the active surface. Stainless steel screw electrodes were placed above the cerebellum as reference electrodes. The tips of the tungsten microelectrodes were moved down in a track at stereotaxic coordinates of A −3.1, L2.7 mm [21]. Recording sites were estimated by reconstructing them using histological analysis on Nissl stained coronal sections.

A differential amplifier (World Precision Instruments DAM50) was used to record extracellular unit activity. The bandwidth was 0.3–10 kHz. Digital data processing was carried out by SPIKE2 for Windows program of (CED1401 data capture system. Sampling rate for unit activity was 20 kHz and samples of 5 min were recorded and processed as described below.

To obtain reliable records, recording started when mean firing frequency and wave shapes of the spikes were unchanged for 20 min. After one control record, the perfusion fluid was changed to ACSF containing uridine or adenosine. Because of the dead space of dialysis probes, recording was started after a certain delay measured individually for each probe. Two samples of multiunit activity were recorded during uridine or adenosine injection, one started 5 min and the other 15 min after the estimated beginning of outflux of compounds. The perfusion fluid was than changed back to ACSF and an additional sample was recorded after a 40 min recovery period.

*Data analysis:* Spikes were selected by setting the discrimination level three times higher than the noise. Spikes were clustered by wave shapes. When clusters were overlapping, mean spike frequency was calculated using total activity. In some hippocampal recordings, clusters of single unit activity patterns were separated successfully. The separated units were assumed to be pyramidal cells because the firing rate was low and complex discharges were observed. Complex spikes were replaced by the first spike in the mean frequency analysis. When the difference in firing rates between control and recovery records was > 20%, data were cancelled. On the basis of statistical variation in mean frequencies of control records, 30% change in mean firing frequency was established as a minimum criteria for uridine responding cell.

All statistical tests were performed by Statistica

for Windows 5.0 software (StatSoft Inc.). In experiments on uridine and adenosine release, average control values were calculated from control samples for each individual rat. Normalized data were compared with control values by Student's *t*-test for correlated samples. Changes were regarded significant at $p < 0.05$.

## Results

*Release of uridine and adenosine in response to kainate induced depolarization:* The perfusion of 5 μM kainate through microdialysis probes in the dorsal hippocampus of six rats significantly increased the extracellular concentration of uridine from $0.88 \pm 0.12$ μM to $2.03 \pm 0.47$ μM and the extracellular concentration of adenosine from $0.65 \pm 0.14$ μM to $1.35 \pm 0.17$ μM. Similarly, the perfusion of 5 sμM kainate through microdialysis probes in the thalamic VPL/VPM nuclei of three rats significantly increased the extracellular concentration of uridine from $0.74 \pm 0.10$ μM to $1.52 \pm 0.41$ μM and the extracellular concentration of adenosine from $0.81 \pm 0.18$ μM to $1.81 \pm 0.34$ μM. After the cessation of kainate administration the uridine and adenosine concentrations returned to control values. TTX had no significant effect on kainate-induced release in the hippocampus and in the thalamus.

*Estimation of effective concentration of uridine:* The extracellular concentration of uridine injected by microdialysis probe was estimated in experiments with double microdialysis probes in the hippocampus. Perfusion of 10 mM uridine through the injection probe for 60 min increased the level of uridine at the test probe from $0.87 \pm 0.06$ μM to $51.5 \pm 7.3$ μM in the first 30 min and to $117.6 \pm 41.7$ μM in the second 30 min. During the recovery period after the wash-out, the estimated uridine concentration was $20.7 \pm 7.3$ μM in the first 30 min and $3.3 \pm 1.1$ μM in the second 30 min. Thus, we estimated a nucleoside concentration of 50–100 μM at the microelectrodes in electrophysiological studies when 10 mM uridine or adenosine was applied. Similarly, the extracellular uridine concentration in the recovery recording period was around 3.3 μM.

Double probes did not induce more severe tissue damage than single probes did as shown by Nissl-stained coronal sections.

*The effect of uridine on the hippocampal and thalamic multiple unit activity:* In the dorsal hippocampus, 14 recording sites of 13 anaesthetized rats were analysed, four of which were excluded from the analysis because of improper recovery. The average frequency of multiunit activity was $66 \pm 10$ spike/s in the dorsal hippocampus. Perfusion with uridine decreased the mean frequency of firing in all of the recording sites (Fig. 1C): to $56.6 \pm 5.4\%$ and $54.5 \pm 5.9\%$ of the control in the first and second recording during uridine administration, respectively. Recordings were usually performed in the pyramidal cell layers as shown in Fig. 1C.

In VPL/VPM, 15 recording sites were examined, five of which were excluded from the analysis because firing rate was instable. The mean spike frequency was $116 \pm 25$ spike/s. Perfusion with 10 mM uridine increased the discharge rates at five recording sites ($144.0 \pm 11.5\%$ and $147.6 \pm 8.1\%$ in the two recording periods, respectively), decreased the discharge rates at two sites ($65.8 \pm 25.0\%$ and $43.8 \pm 10.5\%$ in the two recording periods, respectively) and there was no effect in four sites (Fig. 1C). When 10 mM adenosine was applied, $< 10\%$ activity remained (Fig. 1A) in the four hippocampal and four thalamic recording sites where the test was done.

*Uridine inhibits activity of putative hippocampal pyramidal cells:* Firing of putative pyramidal cells were successfully separated from the multiunit activity in three hippocampal recordings. The cells fired slowly (1.0, 1.2 and 2.5 spike/s, respectively). Series of 2–10 negative–positive action potential bursts characterized by short interspike intervals (~5 ms), dropping amplitudes and increasing spike durations were observed (Fig. 1B). The duration of the negative component of the first action potential was 0.3–0.5 ms. This type of complex spike pattern is characteristic for pyramidal cells [18,23]. Uridine decreased the firing rate of pyramidal cells to 0.1 spike/s (Fig. 1A). The pattern of complex spikes remained unchanged. During the recovery period after the uridine was washed out, neuronal firing rate returned to control values (1.2, 1.3 and 2.2 spike/s, respectively).

*Increased uridine level does not alter the extracellular levels of other nucleosides:* High uridine concentration in the perfusion solution makes chromatographic analysis of nucleosides impossible from samples collected by the injecting probe due to column overload. Thus, effects of increased uridine level on extracellular concentrations of nucleosides (hypoxanthine, xanthine, 2′-deoxycytidine, 2′-deoxyuridine, inosine, guanosine, thymidine and adenosine) were measured in double probe experiments in the hippocampus. Perfusion of 10 mM uridine for 60 min through the injecting probe did not change the concentrations of any nucleosides in the perfusate collected on the test probe. In contrast, when

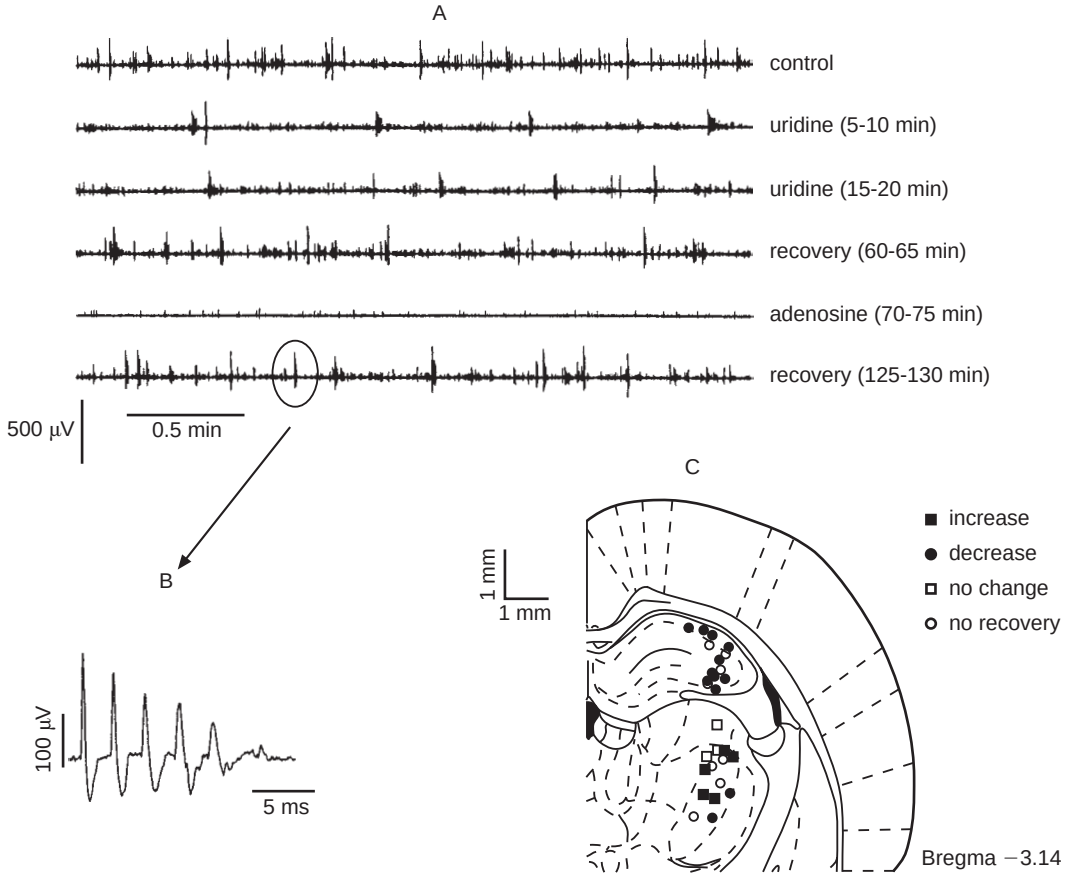


FIG. 1. Effects of uridine and adenosine on hippocampal and thalamic unit activity. (**A**) Samples of unit activity in the hippocampus recorded before, during and after uridine application. It also shows the effect of adenosine and recovery after adenosine application. The time of recording the sample after application of uridine is indicated in brackets. (**B**) A complex spike pattern subtracted from hippocampal multiunit activity. (**C**) The anatomical location of recording sites on the basis of the stereotaxic coordinates. Symbols indicate the different types of effect of uridine on multiunit activity.

10 mM adenosine was perfused for 60 min, the extracellular concentrations of hypoxanthine and inosine showed a $>10$-fold increase while uridine remained unchanged.

## Discussion

*Uridine and adenosine are released by sustained depolarization:* In kainate-induced sustained depolarization a marked, TTX-insensitive elevation in extracellular uridine and adenosine levels was observed. The extent of release was similar in the hippocampus and in the thalamus. Release of adenosine during long-lasting depolarization has already been demonstrated in various parts of the nervous system [5–7], but our study is the first report on depolarization-induced uridine release. Since the applied kainate concentration is below the cytotoxic level [24,25], the observed uridine release is not the result of cell death. However, it could be a component of protective mechanisms induced by sustained depolarization.

*The effect of uridine on the firing rate of hippocampal and thalamic neurones:* Uridine reduced the firing rate of hippocampal neurones to the half of the control value. Decrease in mean firing rate can be achieved by a general decrease in activity of all cell types in the hippocampus, or by a cell type-specific inhibitory effect of uridine. The almost complete block of firing pattern of three individual putative pyramidal cells in the hippocampus in response to uridine suggests that pyramidal neurones are more sensitive to the inhibitory action of uridine than are interneurones. In contrast to uridine-induced partial inhibition, adenosine almost completely inhibited the neuronal activity in the hippocampus, suggesting that it affects both pyramidal cells and interneurones.

In the thalamic VPL/VPM nuclei, a considerable

proportion of cells was insensitive to uridine, while others increased or decreased their firing rates. This suggests that uridine-sensitive cells in the brain have regional differences and that uridine may have different types of mechanisms of action including inhibition and excitation.

*Cellular target of uridine:* Uridine must have a receptor or a modulatory site to bind on neurones. Putative targets involve recently cloned UTP receptors [3,9,12] and adenosine receptors; however, our results raise the question whether uridine has its own specific receptor.

There is a possibility that it was not uridine but some other nucleosides to which uridine is metabolized which induced inhibition after uridine release. In our study, however, uridine did not elevate the extracellular concentration of any nucleosides measured when applied in high concentration. Thus, we suggest that uridine but not its derivatives induced inhibition in the hippocampus.

## Conclusion

From the widely accepted criteria of neurotransmitters, uridine showed the depolarization-induced release and modified the firing pattern of neurones. There is currently no known specific uridine receptor and no data on presynaptic uridine release. We suggest that, similar to adenosine [2], uridine is a neuromodulator candidate released by reversed transport and may be involved in protective mechanisms against sustained hypopolarization.

## References

1. Schubert P, Ogata T, Marchini C *et al. Ann NY Acad Sci USA* **825**, 1–10 (1997).
2. Williams M. *Ann NY Acad Sci* **603**, 93–107 (1990).
3. Burnstock G. *Neuropharmacology* **36**, 1127–1139 (1997).
4. Zimmermann H. *Prog Neurobiol* **49**, 589–618 (1996).
5. Carswell HV, Graham DI and Stone TW. *J Neurochem* **68**, 240–247 (1997).
6. Craig CG and White TD. *J Neurochem* **60**, 1073–1080 (1993).
7. Pazzagli M, Pedata F and Pepeu G. *Eur J Pharmacol* **234**, 61–65 (1993).
8. Communi D and Boeynaems JM. *Trends Pharmacol Sci* **18**, 83–86 (1997).
9. Heilbronn E, Knoblauch BH and Muller CE. *Neurochem Res* **22**, 1041–1050 (1997).
10. Hiruma H and Bourque CW. *J Physiol (Lond)* **489**, 805–811 (1995).
11. King BF, Neary JT, Zhu Q *et al. Neuroscience* **74**, 1187–1196 (1996).
12. Harden TK, Lazarowski ER and Boucher RC. *Trends Pharmacol Sci* **18**, 43–46 (1997).
13. Honda K, Komoda Y, Nishida S *et al. Neurosci Res* **1**, 243–252 (1984).
14. el Kouni MH, Naguib FNM, Park KS *et al. Biochem Pharmacol* **40**, 2479–2485 (1990).
15. Inoue S, Honda K and Komoda Y. *Behav Brain Res* **69**, 91–96 (1995).
16. Myers CS, Fisher H and Wagner GC. *Pharmacol Biochem Behav* **54**, 749–753 (1995).
17. Kardos J, Kovacs I, Szarics E *et al. NeuroReport* **10**, 1577–1582 (1999).
18. Buzsáki G, Leung LS and Vanderwolf CH. *Brain Res Rev* **6**, 139–171 (1983).
19. Steriade M, McCormick DA and Sejnowski TJ. *Science* **262**, 679–685 (1993).
20. Juhász G, Tarcali J, Pungor K and Pungor E. *J Neurosci Methods* **103**, 131–134 (1989).
21. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. Orlando: Academic Press, 1997.
22. Dobolyi Á, Reichart A, Szikra T *et al. Neurochem Int* **32**, 247–265 (1998).
23. Christian EP and Deadwyler SA. *J Neurophysiol* **55**, 331–348 (1986).
24. Ben-Ari Y, Tremblay E, Ottersen OP and Meldrum BS. *Brain Res* **191**, 79–97 (1980).
25. Nadler JV and Cuthbertson GJ. *Brain Res* **195**, 47–56 (1980).

ACKNOWLEDGEMENTS: We are grateful for Dr Julianna Kardos, Central Research Institute for Chemistry, Group of Neurochemistry for critically reviewing the manuscript. This research was supported by grants MKM FKFP 0137/1997 and OTKA T025749 of the Hungarian Scientific Research Foundation to G.J., 5.D. and A.K.K.

**Received 13 July 1999; accepted 6 August 1999**