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Potassium, Rubidium, and 4-Aminopyridine Effects on the Circadian Running Rhythm in the Hamster

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KLEMFUSS, H. AND D. F. KRIPKE. *Potassium, rubidium, and 4-aminopyridine effects on the circadian running rhythm in the hamster.* PHARMACOL BIOCHEM BEHAV 47(3) 409–412, 1994.—The period of the circadian rhythm of wheel-running was shortened when golden hamsters were given supplemental dietary potassium or rubidium for two weeks. Treatment with 4-aminopyridine, a potassium channel blocker, significantly increased circadian period. These period changes (0.17 h for potassium treatment, 0.14 h for rubidium, and 0.07 h for 4-aminopyridine) support previous data indicating that potassium is a component of the mammalian biological clock mechanism.

Potassium Rubidium 4-Aminopyridine Activity Channel blocker Circadian Rhythm

POTASSIUM transport across membranes may be a component of endogenous biological clocks that generate circadian rhythms. In single cells, invertebrates, and mammals, potassium concentration has been shown to vary with a circadian period that can be synchronized by external stimuli such as dawn or dusk, but varies with an endogenous rhythm during constant environmental conditions (4,21,26,30,34). Transient disturbances of potassium flux, caused either by raising extracellular potassium concentrations or by altering membrane characteristics, produce lasting phase shifts of circadian rhythms in nonvertebrates (11,23,34) and in the rodent brain in vitro (9,13). Several models of circadian rhythm generation have led researchers to propose that potassium flux may be one of the "gears" of circadian clocks (1,6,27,30,33). If potassium is a critical component of mammalian timekeeping, then chronic changes in potassium concentrations or membrane transport might alter the period of circadian rhythms.

Long-term manipulations of potassium transport, either by changing the concentration of potassium itself or by using agents such as rubidium and potassium channel blockers, altered the period of circadian rhythms in nonvertebrates including duckweed (20), the housefly (31), the cockroach (10), and the mollusc (24). In two previous reports, we noted that a diet high in potassium slightly shortened the circadian period of wheel-running (τ) in hamsters maintained in constant darkness (DD), but in neither study was the difference statistically significant when data were analyzed using a semiautomated technique (17,19). Since these studies were published we have reanalyzed these data using visual scoring by a rater blind to treatment conditions. After combining the data sets from the two similar studies, high potassium diet shortened τ significantly from 24.14 ± 0.03 h (mean ± SE) in 51 controls fed 171 mmol potassium/kg of diet to 24.00 ± 0.06 h in 50 hamsters fed 1104 mmol potassium/kg of diet ($p < .05$). The duration of time that hamsters were predominantly active (α) was also measured visually. In this analysis α shortened from 12.9 ± 0.4 h in controls to 11.7 ± 0.3 h in hamsters fed the high potassium diet ($p < .05$).

We have extended these studies using a new protocol to reexamine whether changes in potassium intake alter the period of the wheel-running rhythm in golden hamsters. Effects of the alkali metal rubidium and the nonspecific potassium channel blocking agent 4-aminopyridine (4AP) were also evaluated in separate but similar experiments to examine rhythm effects of other treatments that alter potassium concentration and transport.

In the first experiment, adult male Syrian golden hamsters weighing 110–120 g were maintained in group cages for several weeks under a light–dark 8 : 16 schedule (8 h of light per day). Hamsters were given a control diet containing 171 mmol po-

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tassium/kg (diet TD86299 from Teklad, Madison, WI). Each hamster was then transferred to an individual cage containing a running wheel and housed in a ventilated light-tight cabinet. One week later, lights were turned off for the remainder of the experiment. In DD animal care was accomplished using an infrared light source and viewer, so that no light was visible to hamsters at any time. Food and drinking water were available ad lib.

Following two weeks of adaptation to DD conditions, half of the hamsters were randomly assigned to receive a high potassium diet containing 1104 mmol potassium/kg of diet (TD85191). The other half continued on control diet. For the next two weeks in DD, wheel-running activity was recorded every 5 min by a computer system. The period of the circadian wheel-running rhythm was estimated for each hamster during days 4 to 14 after high potassium treatment using the onset periodogram method, an automated procedure that estimates τ based on the onset of activity (16). The duration of the

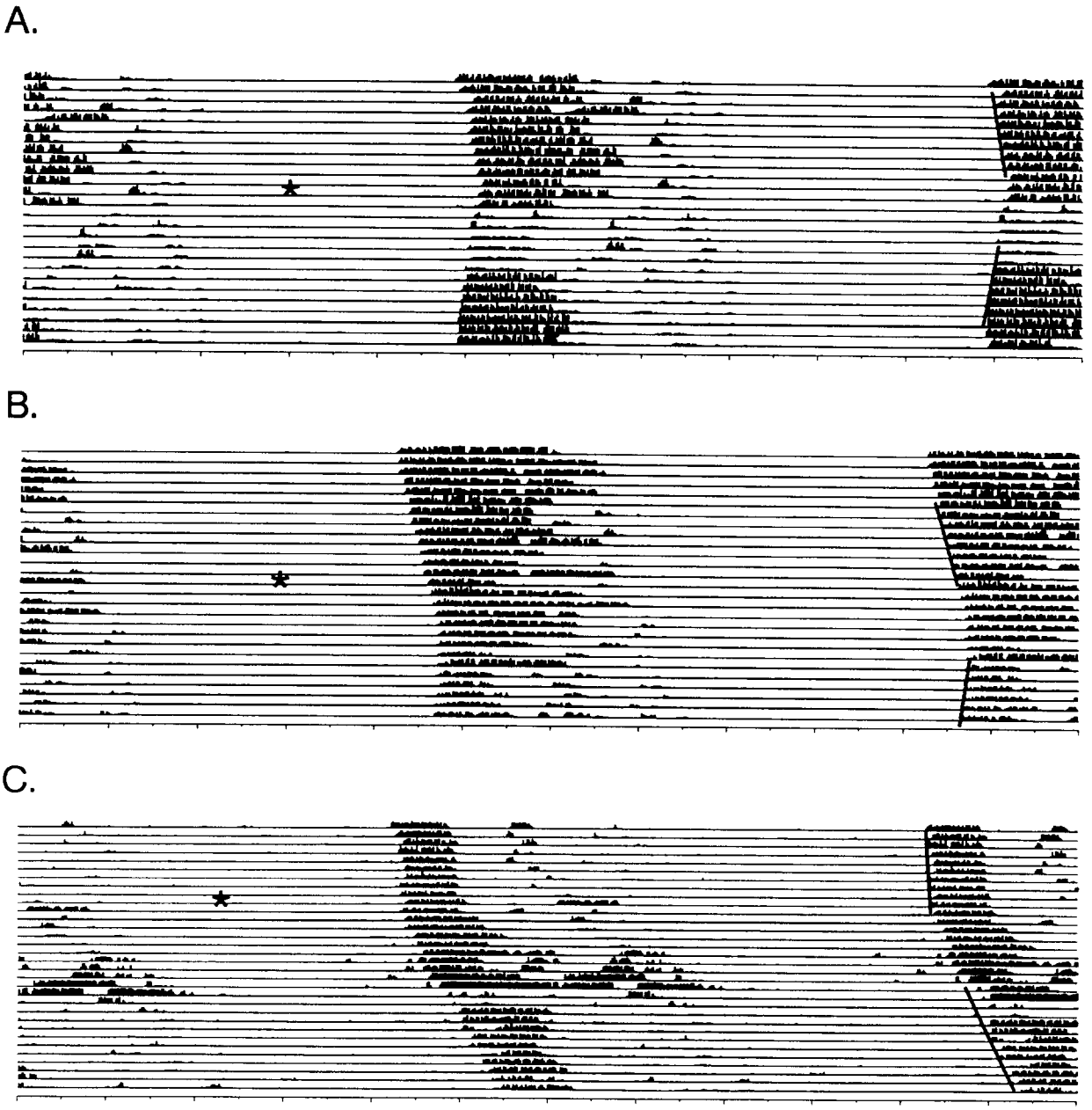

FIG. 1. In these double-plotted actograms, the horizontal axis represents 48 h of wheel-running activity in constant darkness (DD); successive days are plotted vertically. (A) At the asterisk, control diet was replaced by high potassium diet following two weeks of DD. In this animal, circadian period shortened from 24.12 h to 23.95 h, indicated by the black lines on the left side of the plot. (B) Replacement of drinking water with 20 mM RbCl shortened the circadian period of this hamster from 24.12 h to 23.98 h. (C) Substitution of 4AP (0.21 mM) for water increased circadian period in this hamster from 24.05 h to 24.30 h.

wheel-running interval was visually estimated from actograms plotted from the same data. Statistical significance was evaluated using two-tailed unpaired t tests.

The next two experiments followed similar protocols, except that a standard rodent diet (Teklad 4% rodent diet containing 249 mmol potassium/kg diet) was provided to all hamsters. In experiment 2, drinking water was replaced with water containing 20 mM rubidium chloride in half of the running wheel cages after two weeks of adaptation to DD. Similarly, drinking water was replaced with 0.21 mM 4-aminopyridine in half of the cages after two weeks of DD in experiment 3. During days 4 to 14 of drug administration τ and α were measured as in the first experiment.

Figure 1 illustrates effects of these three treatments on circadian period. Potassium and rubidium treatments both significantly shortened the circadian period of wheel-running (Table 1). The potassium channel blocker 4AP had the opposite effect of lengthening τ ($p < .05$). The magnitude of these effects is comparable to the period-lengthening effect of lithium previously described in hamsters (17). Similarly, rubidium and 4AP both influenced activity duration, but in opposite directions. Rubidium decreased α from the control duration of 12.5 ± 0.4 h to 11.4 ± 0.3 h, while 4AP treatment increased α from 12.8 ± 0.2 h to 13.5 ± 0.2 h (mean ± SE, $p < .05$ in each case). There were no significant effects on weight gain, fluid intake, or general health related to any of these treatments.

These new results confirm reanalyses of our previous data, showing that potassium supplementation shortens circadian period. Potassium supplementation did not significantly affect α in this experiment (control α 12.9 ± 0.3 h vs. high potassium diet α 12.4 ± 0.4 h). In the previous studies high potassium diet was provided for three weeks before activity duration was measured, so the present study may have ended before clear effects of potassium diet on α had sufficient time to develop.

The suprachiasmatic nuclei, located in the inferior hypothalamus, contain the primary circadian pacemaker in mammals (25). Although the mammalian brain is generally resistant to alterations in dietary mineral intake, it has been reported that chronic elevation of dietary potassium intake increased brain potassium concentration and cellular uptake of radiolabelled potassium in the hypothalamus (5). The suprachiasmatic nucleus may be especially sensitive to changes in blood composition, since this nucleus is one of the few brain regions labelled following systemic injection of fluorescein, a dye which does not cross the blood–brain barrier (22). Therefore, it is possible that the high potassium diet shortened circadian period by altering potassium transport in the region of the brain controlling circadian rhythmicity.

Since high potassium diet shortened τ by about 10 min, the onset of wheel-running activity was about 2 h earlier in potassium-treated hamsters than in controls after two weeks of DD. This result can be compared to a study in which elevated potassium (20 mM) was infused directly into the suprachiasmatic nucleus of blind rats for two weeks. This treatment disrupted the overt circadian rhythm of drinking, but after the infusion stopped the phase of the drinking rhythm was advanced by about 4 h compared to the expected phase (32). Thus, high potassium, whether taken orally or infused directly into the brain, may advance the biological clock controlling circadian rhythms in rodents.

Chronic treatment with rubidium and 4AP affected both τ and α in these experiments. Rubidium ion equilibrates with and replaces potassium ion in most cellular compartments, but these alkali metal cations have different effects on ion channels and membrane-bound proteins such as Na^+/K^+ATPase (7,31). Interference with transport of the potassium ion may cause some of the central nervous system actions associated with chronic rubidium treatment (12). In the present report, rubidium treatment resembled the high potassium diet, since both treatments shortened circadian period. Rubidium treatment has also been reported to shorten τ in the cockroach (10), increase τ in duckweed (20), and either increase or decrease τ in the housefly (31) and a plant (29). Other reported effects of rubidium treatment include a phase delay and shortened duration of the light-synchronized wheel-running rhythm in hamsters (3), advance of the phase of the body temperature peak relative to motor activity in a woman treated with rubidium as an antidepressant (28), inhibition of the photoperiod-dependent regression of the hamster testes (3,8), and restoration of coherent activity rhythms in hamsters in which morning and evening components had "split" (14). Thus, rubidium affects various aspects of circadian rhythmicity in plants, invertebrates, and mammals, but no clear picture of its actions has yet emerged.

The rhythm effects of potassium and rubidium were opposite to the effects of 4AP, which crosses the blood–brain barrier sufficiently to block potassium channels in the central nervous system (2). Another potassium channel blocker, tetraethylammonium, increased circadian period in the housefly (31) and in preliminary studies in hamsters (18), but shortened τ in the duckweed (21). Tetraethylammonium and 4AP influence several types of potassium channel, particularly at high doses (15), so these studies do not indicate which classes of potassium channel might be involved in altering circadian period and activity duration.

Potassium transport in the suprachiasmatic nucleus may be a component of mammalian biological clocks, but it is not yet possible to infer whether potassium ion is a crucial "gear" of a clock, a "hand" connecting the clock mechanism to overt behavior, or part of the input pathway responsible for synchronizing the clock to stimuli such as light. Since chronic dietary potassium supplementation augmented the phase-advancing effects of light in hamsters (19), brain potassium might influence the action of light on circadian rhythms. The current results suggest that potassium also may have a role in determining the period of the circadian pacemaker in mammals.

TABLE 1

TREATMENT EFFECTS ON τ

	K (Exp. 1)	Rb (Exp. 2)	4AP (Exp. 3)
Control	24.15 ± 0.03 (18)	24.10 ± 0.03 (17)	24.16 ± 0.02 (15)
Treatment	23.98 ± 0.04 (15)	23.94 ± 0.04 (12)	24.23 ± 0.02 (17)
Significance	$p < .01$	$p < .01$	$p < .05$

Mean period of the wheel-running rhythm during days 4 to 14 of treatment, in hours ± SE, with the number of animals used in parentheses. Level of significance calculated by unpaired t test in each separate experiment.

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