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Spinal Antinociceptive Action of Na^+–K^+ Pump Inhibitor Ouabain and Its Interaction with Morphine and Lidocaine in Rats

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Background: **The Na^+,K^+–adenosine triphosphatase is a ubiquitous enzyme system that maintains the ion gradient across the plasma membrane of a variety of cell types, including cells in the central nervous system. We investigated the antinociceptive effect of intrathecally administered ouabain and examined its potential interaction with spinal morphine and lidocaine.**

Methods: **Using rats chronically implanted with lumbar intrathecal catheters, the ability of intrathecally administered ouabain, morphine, and lidocaine and of mixtures of ouabain–morphine and ouabain–lidocaine to alter tail-flick latency was examined. To characterize any interactions, isobolographic analysis was performed. The effects of pretreatment with intrathecally administered atropine or naloxone also were tested.**

Results: **Intrathecally administered ouabain (0.1–5.0 μg), morphine (0.2–10.0 μg), and lidocaine (25–300 μg) given alone produced significant dose- and time-dependent antinociception, but systemic administration of ouabain did not produce such an effect. The median effective dose (ED_{50}) values for intrathecally administered ouabain, morphine, and lidocaine were 2.3, 5.0, and 227.0 μg, respectively. Isobolographic analysis exhibited a synergistic interaction after the coadministration of ouabain and morphine. With ouabain and lidocaine, there was no such evidence of synergism. Intrathecally administered atropine, but not naloxone, completely blocked the antinociceptive effect of ouabain and attenuated its interaction with spinally administered morphine.**

Conclusions: **Intrathecally administered ouabain produces antinociception, at least in part, *via* an enhancement of cholinergic transmission in the spinal nociceptive processing system. The results of the interaction of ouabain with morphine and lidocaine suggest that modulation of Na^+-,K^+-electrochemical gradients and thus subsequent release of neurotransmitters in the spinal cord are likely to play important roles in the spinal antinociceptive effect of intrathecally administered ouabain. (Key words: Acetylcholine; atropine; ion channels; local anesthetics; opioid.)**

OUABAIN is a specific inhibitor of membrane-bound Na^+,K^+-adenosine triphosphatase (Na^+-K^+ pump), which regulates the intracellular Na^+ ($[Na^+]_{in}$) and K^+ ($[K^+]_{in}$) content of a variety of cell types, including in the central nervous system.[1] Moreover, ouabain binding sites have been found in various areas of the rat brain.[2] In nerve and muscle cells, the maintenance of a high $[K^+]_{in}$ and low $[Na^+]_{in}$ is important for the electrical activity of the cell. Schlue[3] reported that the increase in $[Na^+]_{in}$ resulting from inhibition of the Na^+-K^+ pump affects the intracellular Ca^{2+} ($[Ca^{2+}]_{in}$) concentration by stimulating the Na_{in}^+-Ca^{2+} exchange mechanism. The reduced electrochemical gradient for Na^+ and the increased $[Ca^{2+}]_{in}$ concentration can cause release of acetylcholine in the nervous system.[4]

The spinal cord is an important neuronal structure for pain transmission and is the pharmacologic site of action of agents such as opioids,[5,6] local anesthetic agents,[7] and α_2-adrenergic agonists,[8] which are used to provide spinal antinociception in clinical situations. Because intrathecally administered cholinergic agonists and acetylcholinesterase inhibitors produce antinociceptive effects in animals and humans,[9,10,11] the mechanism underlying such analgesic actions could involve the release of acetylcholine at the spinal cord level.[12] Moreover, recent studies seem to suggest that cholinergic transmission at the spinal cord level is of relevance to opioid-mediated analgesia.[5,6] For example, intravenously ad-

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ministered morphine increased the concentration of norepinephrine and acetylcholine in cerebrospinal fluid,[6] and the antinociception resulting from administration of morphine is inhibited by intrathecally administered atropine.[5] Several studies also have documented that antinociception attributable to systemic or intrathecal administration of opioids is enhanced by intrathecal administration of acetylcholinesterase inhibitors.[10,13] Thus, there could be potential for an interaction between the effects of ouabain and those of morphine on nociceptive processing.

The activity of the Na^+-K^+ pump is responsible for generating and maintaining electrochemical gradients across the membrane *via* the active pumping of three Na^+ out of and two K^+ into the cell. Because local anesthetic agents block the generation of neural action potentials and their propagation by a selective effect on Na^+ channels of neuronal membranes and K^+ channels as well,[7] especially blocking Na^+ influx through Na^+-selective pores, there could be a significant interaction between the effects of ouabain and those of local anesthetic agents. In the current study, on conscious rats, we examined (1) whether intrathecally administered ouabain produces antinociceptive effects, and (2) whether it modulates the antinociceptive actions of spinally administered morphine and lidocaine on somatic nociception.

Materials and Methods

Animals

With approval from our Animal Care and Use Committee, studies were performed on male Sprague-Dawley rats weighing 250–350 g. Rats were housed individually in a temperature-controlled (21 ± 1°C) room with a 12-h light/dark cycle, and they were given free access to water and food. All surgical procedures were performed with the rats during intraperitoneally administered midazolam- (2 mg/kg) and ketamine- (40 mg/kg) induced anesthesia. Using the method described by Yaksh and Rudy,[14] an intrathecal catheter (PE-10, 8.5 cm) was inserted through an opening in the cisterna magna to the lumbar subarachnoid space. The external arm of the catheter was tunnelled subcutaneously to emerge at the neck. After surgery, the rats were again housed individually and allowed to recover for 1 week before the administration of drugs.

Each animal was studied two or three times in an experimental series, with a 2–4-day intervals between studies. After experimental use, each rat was killed with an overdose of pentobarbital, and an injection of 1% methylene blue was given to confirm the position of the catheter and the likely spread of the injectate.

Nociceptive Test

Nociceptive threshold was assessed using the tail-flick test. In the tail-flick test, the response to a noxious somatic stimulus was measured by monitoring the latency to withdrawal from the heat source (a 50-W projection lamp bulb, KN-205E; Natsume, Tokyo, Japan) focused on the dorsal surface of the tail. The same portion of the tail was exposed to the stimulus in each test. The mean (range) baseline value for tail-flick latency was 3.5 s (3.3–3.8 s). A cut-off time of 10.0 s was imposed to minimize damage to the skin of the tail during the experiment. Tail-flick latencies were determined 5, 10, 15, 20, 30, 40, 50, and 60 min after intrathecal administration of drugs. The effects of ouabain alone when given by intraperitoneal injection and of intrathecal pretreatment with naloxone or atropine also were tested 10 min before intrathecal administration of ouabain, morphine, or a mixture of the two.

Motor blockade was graded according to the scale proposed by Langerman *et al.*[15] for rabbits, which we modified for the rat model as follows: 0 = free movement of hindlimbs without limitation; 1 = limited or asymmetrical movement of the hindlimbs to support the body and walk; 2 = inability to support the back of the body on the hindlimbs, with detectable ability to move the limbs and respond to a pain stimulus; and 3 = total paralysis of the hindlimbs.

Drugs and Injections

The drugs administered in the experiments were ouabain octahydrate (molecular weight [MW] 363.8; Sigma Chemical Co., St. Louis, MO), morphine hydrochloride (MW 321; Sankyo, Tokyo, Japan), lidocaine hydrochloride (MW 270.8; Sigma), naloxone hydrochloride (MW 363.8; Sigma), and atropine sulfate injection (MW 694.8; Danabe, Osaka, Japan). All drugs were dissolved in normal saline, with *p*H levels of ≈7.0. Each animal was placed in an individual plastic cylinder with an opening to allow the tail to protrude. After baseline measurements for tail-flick latency had been obtained, each animal received an intrathecal injection of ouabain (0.1, 0.25, 0.5, 1.0, 2.0, or 5.0 μg), morphine (0.2, 0.5, 1.0, 2.0, 5.0, or 10.0 μg), or ouabain plus morphine. Physiologic saline (20 μl, *p*H 6.5) served as a control. To assess the antinociception produced by Na^+ channel

blockade, the effects of lidocaine (25, 50, 100, 200, or 300 μg) alone and those of a lidocaine–ouabain combination were studied. The effect of intraperitoneal injection of ouabain (5 and 30 μg/kg) also was examined. All drugs were administered in a total volume of 10 μl followed by 10 μl saline to flush the catheter.

Cardiovascular Variables

To examine whether any changes in cardiovascular variables might have occurred during the experiments with ouabain, arterial blood pressure and heart rate were measured before and after intrathecal injection of ouabain (2 μg) in the five animals using a noninvasive blood pressure monitor (MK-1030, Muromachi Kikai Co., LTD., Tokyo, Japan).

Statistical Analyses

All data are presented as mean ± SD. The response in the tail-flick test is expressed as the percentage of the maximum possible effect (%MPE), where %MPE = (Post-drug tail-flick latency − Baseline tail-flick latency)/(10 s − Baseline tail-flick latency) × 100. The effects of drugs on tail-flick latency, mean arterial blood pressure, and heart rate were evaluated for linearity and deviation from parallelism by a one-way analysis of variance and Fisher's protected least significant difference test. Other comparisons between groups were analyzed using a two-way analysis of variance and Scheffé's F test. The motor scores, confidence intervals, and the area under the time–response curve were evaluated for statistical significance with a Student's *t* test. A probability value <0.05 was considered statistically significant. In addition, the time course for the effect expressed as the area under the time–response curve was calculated by a trapezoidal rule.[10] Median effective dose (ED_{50}) values and 95% confidence intervals were calculated using a least-squares linear regression model in which log dose values were used. Isobolographic analysis of the ouabain–morphine and ouabain–lidocaine interactions was conducted in accordance with procedure of Tallarida *et al.*[16]

Results

Dose- and Time–Response Analysis

Individual Drug Responses. Intrathecal administration of ouabain (0.1–5.0 μg) alone produced a significant dose-dependent antinociception in the tail-flick test (fig. 1). The peak effects of ouabain were observed 5 min after administration of drug. With 0.25 and 0.50 μg ouabain, the tail-flick latency increased once 5 min later and then decreased, a significant change with 0.5 μg ($P < 0.05$) 30 and 40 min after the administration. The tail-flick latency was not measured in those animals that received the highest dose of 5 μg ouabain administered intrathecally, because such animals appeared to become unstable (tonic convulsive behavior, restless movements) ≈20 min after administration. Such behavior lasted for 20–120 min. With doses of ouabain of 0.1, 0.25, 0.5, 1.0, and 2.0 μg, these adverse effects were not noted in the 60–90 min after the intrathecal injection.

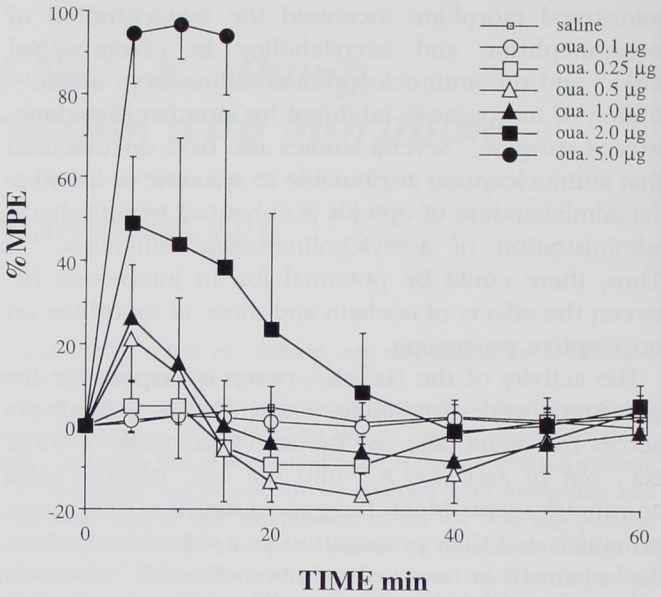

Fig. 1. Time course of the antinociceptive effect (%MPE) of intrathecally administered ouabain in tail-flick tests. Each point represents the mean ± SD from five or six rats.

Intrathecal administration of morphine and lidocaine produced antinociceptive effects in the tail-flick tests that were time- and dose-dependent (fig. 2). The peak effects of morphine and lidocaine were observed 15 min and 5 min after administration of drug, respectively.

Responses to Drug Combinations. In contrast to moderate doses of morphine (2.0 μg) and ouabain (1.0 μg), the concomitant administration of the drugs produced a significant prolongation of the tail-flick latency (figs. 3 and 4). Figure 3 illustrates effects of combinations of morphine and ouabain at doses in a 2:1 ratio and shows that concomitant administration of ouabain and morphine produced significant dose-dependent antinociception (*i.e.*, increase in tail-flick latency). When lidocaine was given intrathecally with ouabain (fig. 4), no significant increase in the %MPE was observed (com-

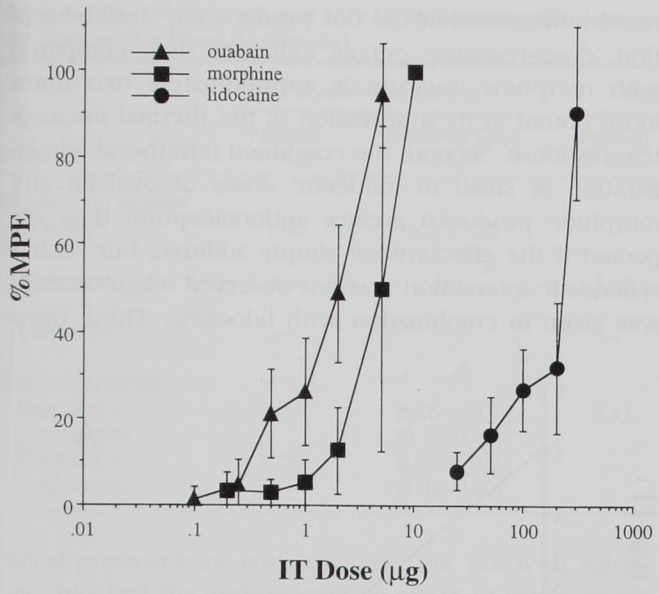

Fig. 2. Log dose–response curves for the effects of intrathecally administered ouabain, morphine, and lidocaine on the thermal nociceptive threshold. Data are plotted as %MPE *versus* log dose in micrograms. Each point represents the mean ± SD from five or six rats.

pared with the effects of the same doses of lidocaine given alone).

Isobolographic Analyses

To assess the antinociceptive interaction of intrathecally administered ouabain–morphine and ouabain–lidocaine, isobolographic analyses were performed (fig. 5). The ED_{50} values (with 95% confidence intervals) for the effects of these drugs on tail-flick latency were: ouabain, 2.3 μg (1.7–3.1); morphine, 5.0 μg (2.7–7.4); and lidocaine, 227 μg (176–277) when they were administered intrathecally alone. The experimentally determined ED_{50} values for the drugs in combination were 0.26 μg (0.12–0.40) for ouabain and 0.54 μg (0.25–0.83) for morphine. The expected additive ED_{50} values were calculated to be 1.18 μg (0.84–1.53) for ouabain and 2.50 μg (2.05–2.94) for morphine. Thus, the combined effect of ouabain and morphine indicated a synergistic interaction, the experimental doses being significantly lower than the doses indicating a purely additive interaction ($P < 0.01$; fig. 5A and table 1). In contrast, the experimentally determined ED_{50} values were 131 μg for lidocaine and 0.65 μg for ouabain. The expected additive ED_{50} values were calculated to be 152 μg for lidocaine and 0.75 μg for ouabain (fig. 5B and table 1). Although numerically less, the confidence intervals of the points overlap, and the fractional analysis (0.86) does not differ significantly from 1 (table 1).

Antagonism Produced with Intrathecally Administered Atropine and Naloxone

Intrathecal pretreatment with naloxone (10 μg) antagonized the antinociceptive effects of morphine (5 μg) and did not affect the changes in tail-flick latency obtained with ouabain (2 μg; data not shown). In contrast, atropine (5 μg) antagonized the antinociceptive effect of intrathecally administered ouabain (2 μg) and attenuated the effect of a combination of intrathecally administered morphine (2 μg) and ouabain (1 μg) (fig. 6). This dose of atropine did not produce any effect on tail-flick latency when administered alone (data not shown).

Other Effects

When ouabain (5 or 30 μg/kg) was administered intraperitoneally, there was no prolongation of tail-flick latency (data not shown), and no change in behavior was noted in any of the rats. Animals given ouabain (2 μg)

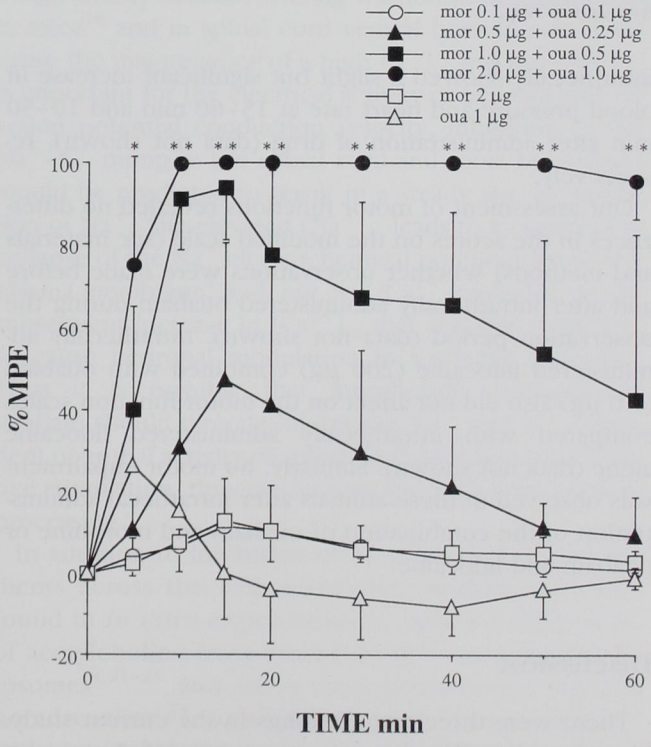

Fig. 3. Time–effect curves for various mixtures of morphine (mor) and ouabain (oua) in tail-flick tests. The combination of 2.0 μg morphine and 1.0 μg ouabain produced a significant prolongation of tail-flick latency. *$P < 0.05$ or **$P < 0.001$ compared with the baseline preadministration values. Each point represents the mean ± SD from five or six rats.

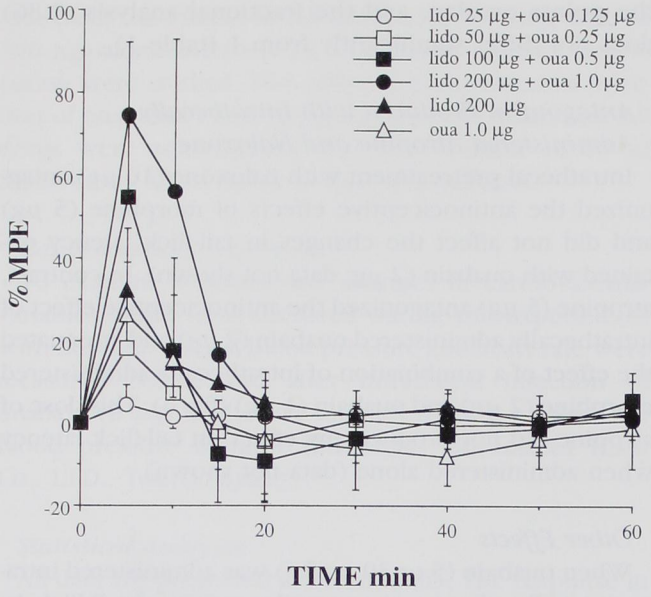

Fig. 4. Time–effect curves for various mixtures of lidocaine (lido) and ouabain (oua) in tail-flick tests. In contrast to 200 μg lidocaine and 1.0 μg ouabain, the concomitant administration of the drugs did not produce a significant prolongation of tail-flick latency. Each point represents the mean ± SD from five or six rats.

intrathecally showed a slight but significant increase in blood pressure and heart rate at 15–60 min and 10–50 min after administration of drug (data not shown), respectively.

Our assessment of motor functions revealed no differences in the scores on the modified scale (see materials and methods) whether observations were made before and after intrathecally administered ouabain during the observation period (data not shown). Intrathecally administered lidocaine (200 μg) combined with ouabain (1.0 μg) also did not affect on the motor function scales compared with intrathecally administered lidocaine alone (data not shown). Similarly, no motor impairment was observed in these animals after intrathecal administration of the combination of ouabain and morphine or ouabain and lidocaine.

Discussion

There were three main findings in the current study. First, intrathecally administered ouabain (1–5 μg) produced a significant dose- and time-dependent antinociceptive effect in tests using noxious thermal stimulation, although a smaller intrathecal dose of ouabain (0.5 μg) produced a delayed hyperalgesic state. Although its systemic administration did not produce any antinociception, dose-response curves indicated that, compared with morphine, ouabain is approximately two times more potent in its suppression of the thermal nociceptive response. Second, the combined intrathecal administration of small to moderate doses of ouabain and morphine produced greater antinociception than expected if the effects were simply additive, but such a synergistic interaction was not observed when ouabain was given in combination with lidocaine. Third, intra-

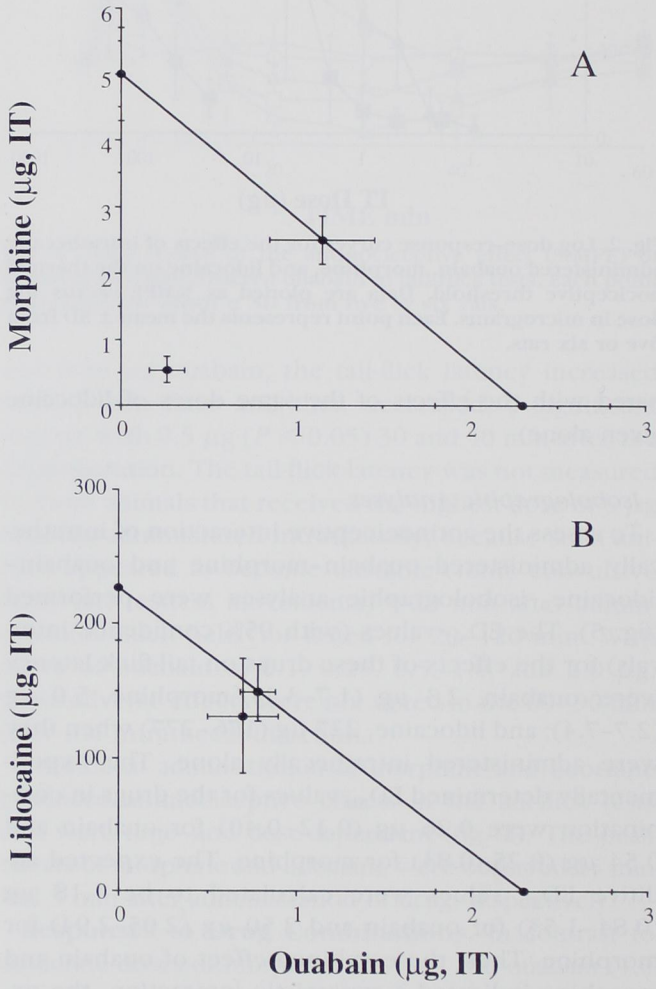

Fig. 5. ED_{50} isobologram for the interaction of the antinociceptive effects of intrathecally administered morphine–ouabain (*A*) and lidocaine–ouabain (*B*) mixtures when coadministered in a fixed-dose ratio. The straight line connecting the single-drug ED_{50} points is the theoretical additive line, and the point shown on this line is the theoretical additive ED_{50} point. The experimental point for the morphine–ouabain mixture was significantly ($P < 0.01$) below the additive line, indicating a synergistic effect. The experimental point for the lidocaine–ouabain mixture was not significantly below the additive line. Each point represents the mean ± SD.

Table 1. ED_{50} Values ± SD and 95% CI for Intrathecally Administered Ouabain, Morphine, and Lidocaine (Either Alone or in Mixtures with a Fixed-dose Ratio)

	Ouabain Component		Morphine Component		Lidocaine Component		
Group	Fraction of ED_{50}	Intrathecal Dose (μg)	Fraction of ED_{50}	Intrathecal Dose (μg)	Fraction of ED_{50}	Intrathecal Dose (μg)	Sum of ED_{50} Fractions
Single-drug studies							
Ouabain	1.00	2.3 ± 0.6 (1.7–3.1)	—	—	—	—	1.00
Morphine	—	—	1.00	5.0 ± 0.7 (2.7–7.4)	—	—	1.00
Lidocaine	—	—	—	—	1.00	2.27 ± 41 (176–277)	1.00
Interaction studies							
Ouabain + morphine	0.11	0.26 ± 0.1 (0.12–0.40)	0.11	0.54 ± 0.2 (0.25–0.83)	—	—	0.22
Ouabain + lidocaine	0.28	0.65 ± 0.2 (0.38–0.92)	—	—	0.58	131 ± 43 (78–184)	0.86

thecal pretreatment with atropine, but not with naloxone, blocked the antinociceptive effect of intrathecally administered ouabain and decreased the antinociceptive response produced by the combined intrathecal injection of ouabain and morphine. These results may lead to a greater understanding of pain management.

The current study is the first demonstration of an inhibition of nociceptive responses by intrathecal administration of ouabain. Ouabain is a selective block of the plasma membrane Na^+-K^+ pump,[1] and thus the electrophysiologic consequences should produce small fiber depolarization[17] of spinal cord and roots when given in the subarachnoid space. In addition, ouabain binding sites have been found in many regions of the brain and a high-affinity ouabain binding was found in spinal roots in mice[18] and in spinal cord ventral horn in rats.[19] Because the maintenance of a high $[K^+]_{in}$ and low $[Na^+]_{in}$ is important for the electrical activity of neurons and for action potential conduction velocity, inhibition of the Na^+-K^+ pump in the spinal cord and roots by ouabain would be predicted to result in a steady net accumulation of Na^+. The elevated $[Na^+]_{in}$ leads to a consequent collapse of the Na^+ electrochemical potential across the plasma membrane[4] and, *via* Na^+/Ca^{2+} exchange, to the subsequent increase in $[Ca^{2+}]_{in}$ that could be sufficient to cause neuronal modulation in excitable neurons.[4] Thus it is possible that intrathecally administered ouabain should directly inhibit the Na^+- and K^+-dependent neuronal activity of spinal cord neurons to nociceptive stimulation, thus modulating the spinal nociceptive processing.

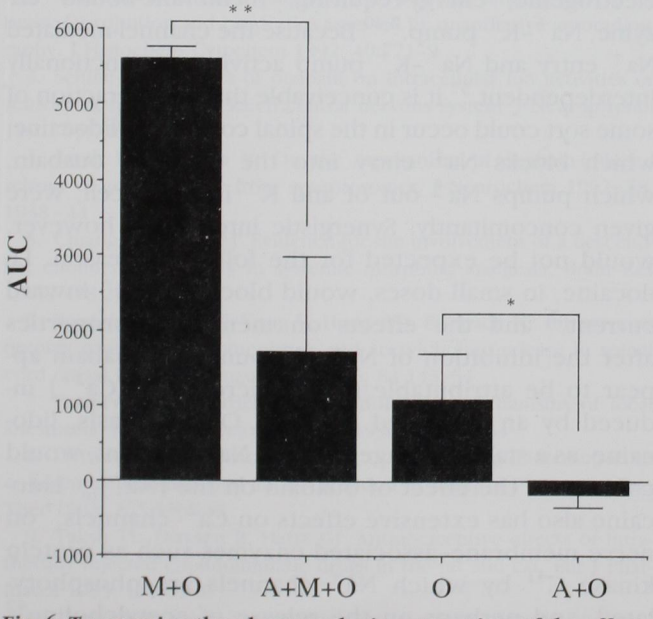

Fig. 6. To examine the pharmacologic antagonism of the effects of ouabain (O; 2 μg) and ouabain (1 μg)–morphine (M; 2 μg), atropine (A; 5 μg) was administered intrathecally 10 min before the administration of the agonists. Each bar represents the mean ± SD from five rats. *P < 0.05; **P < 0.01. AUC = area under the time–response curve.

In addition to inhibition of the electrochemical gradients across the cell membrane, ouabain has been found in *in vitro* experiments to increase the release of acetylcholine from cortex slices[20] and from synaptosomes[4,21–23] and other neurotransmitters such as noradrenaline,[24] serotonin (5-hydroxytryptamine),[25] and γ-aminobutyric acid[26] in brain slice preparations. Those substances all affect or modulate pain transmission.[8–10,27,28] The reduction in the Na^+ electrochemical gradient should directly inhibit the Na^+-dependent neuronal activity necessary for the reuptake of

transmitters, leading to a reduction of neurotransmitter stores.[29] Ouabain, by elevating the background level of $[Ca^{2+}]_{in}$, may enhance spontaneous and evoked neurotransmitter release.[4,29] Ouabain caused a dose-dependent increase in release of acetylcholine in synaptosomes,[4] effects that could be attributable to the increment of $[Ca^{2+}]_{in}$ resulting from accumulation of $[Na^+]_{in}$ by inhibiting Na^+-K^+ pump, but also to mechanisms independent of the changes in ionic distribution.[4,22] Although we cannot exclude the possibility that the antinociceptive action of ouabain is largely attributable to Na^+-K^+ pump inhibition *per se,* it is possible that ouabain acts, at least in part, *via* increased neurotransmitter release at the spinal cord level. The finding that intrathecal pretreatment with atropine antagonized ouabain-induced antinociception could provide evidence that ouabain could produce its antinociceptive effect through an action, perhaps, at specific muscarinic receptors within the spinal cord.

The explanation for the hyperalgesia produced by a small intrathecal dose of ouabain is not clear. One possibility is that the increase of $[Na^+]_{in}$ and $[Ca^{2+}]_{in}$ that follows inhibition of the Na^+-K^+ pump may facilitate the neuronal conduction of action potentials. Such an increase in $[Na^+]_{in}$ and $[Ca^{2+}]_{in}$ could further increase the membrane permeability and thus could facilitate the regenerative process of Na^+ and Ca^{2+} channel openings.[30] It has been described that low concentrations of ouabain at 10^{-7} M actually can stimulate the Na^+-K^+ pump.[17] An increase in $[Na^+]_{in}$ and $[Ca^{2+}]_{in}$ and a potential increased production of endogenous nitric oxide in the spinal cord by ouabain[31] might lead to hyperalgesia.

Another important finding of the current study was that intrathecally administered ouabain acted synergistically to potentiate the antinociceptive action of spinally administered morphine. Although any discussion of the mechanism underlying the observed synergism would be speculative at this stage, the current results suggest that ouabain and morphine act, at least in part, through the release of acetylcholine to produce analgesia. Synergistic interactions can occur when drugs affect different critical points along a common pathway.[32] Intrathecally administered atropine, although it did not *per se* exert an endogenous steady-state effect on nociceptive transmission, can reverse the antinociceptive effect of morphine,[5,6] which produces a dose-dependent increase in concentrations of acetylcholine and norepinephrine in cerebrospinal fluid.[6] Because the main electrophysiologic action of an opioid such as morphine is thought to involve hyperpolarization of the neuronal membrane attributable to the opening of K^+ channels,[33,34] one possibility is that the synergistic effect of ouabain with morphine on spinal antinociception might be *via* an effect on K^+ channels through its inhibition of Na^+-K^+ pump.[35] We found, however, that an effective blockade of ouabain-induced antinociception and an attenuation of the antinociceptive effect of ouabain–morphine followed pretreatment with atropine. This suggests that such synergistic interaction is likely, at least in part, to be attributable to subsequent change in neurotransmitter release, rather than electrochemical gradients of K^+ *per se,* involved in nociceptive processing within the spinal cord.

Local anesthetic agents block action potential generation and propagation by interacting with individual Na^+ channels and converting the channel from an open, resting, or closed state to an inactive state.[7] Lidocaine directly suppresses dorsal horn neuronal activity of the spinal cord to noxious thermal stimulation in a dose-related manner.[36] Several reports indicate that lidocaine given intrathecally interacts synergistically with morphine,[37] Ca^{2+} blockers,[38] and clonidine[39] in animals. In neuronal cells, as is well known, the most important ionic disequilibria are created and maintained by the electrogenic, energy-requiring, membrane-bound enzyme, Na^+-K^+ pump.[7,40] Because the channel-mediated Na^+ entry and Na^+-K^+ pump activity are functionally interdependent,[37] it is conceivable that an interaction of some sort could occur in the spinal cord when lidocaine, which blocks Na^+ entry into the cell, and ouabain, which pumps Na^+ out of and K^+ into the cell, were given concomitantly. Synergistic interaction, however, would not be expected for the following reason. Lidocaine, in small doses, would block the Na^+ inward current,[7] and the effects on membrane properties after the inhibition of Na^+-K^+ pump by ouabain appear to be attributable to the increase in $[Ca^{2+}]$ induced by an increased $[Na^+]_{in}$.[3] On this basis, lidocaine, as a stabilizing agent of the Na^+ gradient, would counteract the effect of ouabain on the $[Na^+]_{in}$. Lidocaine also has extensive effects on Ca^{2+} channels,[7] on nerve membrane–associated enzymes such as protein kinase C[41] by which Na^+ channels are phosphorylated, and perhaps on the release of acetylcholine[42] and is responsible for a reduction in the amount of neurotransmitters released during depolarization. Lidocaine could reverse ouabain-induced inhibition of glutamate uptake in rat synaptosomes.[43] It is thus

conceivable that suppression of neuronal transmission signals by lidocaine might offset the action of ouabain. In addition, because we administered ouabain and lidocaine concomitantly in a fixed dose, we cannot exclude the possibility that different interactions could occur in different doses or timing of administration. These issues remain to be investigated in further studies.

The current study demonstrates that intrathecal injection of ouabain, a Na^+-K^+ pump inhibitor, produces predominantly a spinal antinociceptive effect in the tail-flick test (although hyperalgesia was noted with a small dose). Our results suggest that the antinociceptive effect is attributable to the enhancing effect of the reduced Na^+ electrochemical gradient on release of acetylcholine. The synergistic effect observed after coadministration of ouabain and morphine is suggestive of a functional interaction at the spinal level in the nociceptive processing system between such an increase in release of acetylcholine and opioid receptor activation.

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