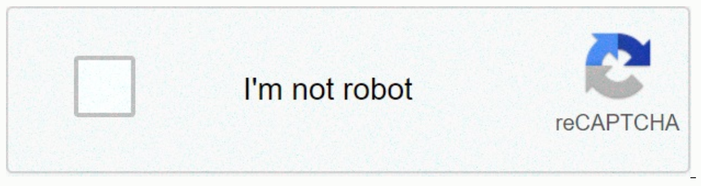

Continue

Bumetanide davis drug guide pdf

1 Department of Life Sciences, National Taiwan Normal University, Taipei, Taiwan, Find Articles Meng-Chang Ko 1 Department of Life Sciences, National Taiwan Normal University, Taipei, Taiwan, Find Articles Ming-Chung Lee 1 Department of Life Science, National Taiwan Normal University, Taipei, Taiwan, Find Articles Tso-Hao Tang 2 Laboratory of Experimental Models of Neurodegenerative Processes, Federal State Budget Scientific Institution Scientific Research Institute of Physiology and Basic Medicine (SRIPhBM), Novosibirsk, Russia, 3 Novosibirsk State University, Novosibirsk, Russia, Find Articles Tamara G Amstislavskaya 2 Laboratory of Experimental Models Neurodegener Processes, Federal State Budget Scientific Institution Scientific Research Institute of Physiology and Basic Medicine (SRIPhBM), Novosibirsk, Russia, 3 Novosibirsk State University, Novosibirsk, Russia, Find Articles Maria A Tikhonova 4 Department of Biochemical Science and Technology , National Chiayi University , Chiayi, Taiwan, Find Articles Yi-Ling Yang 1 Department of Life Science, National Taiwan Normal University, Taipei, Taiwan, Find Articles Kwok-Tung Lu 1 Institute of Life Science, National Taiwan Normal University, Taipei, Taiwan, 2 Laboratory of Experimental Models of Neurodegenerative Processes, Federal State Budget Scientific Institution Scientific Research Institute of Physiology and Basic Medicine (SRIPhBM), Novosibirsk, Russia, 3 Novosibirsk State University, Novosibirsk, Russia, 4 Institute of Biochemical Science and Technology, National Chiayi University, Chiayi, Taiwan, Yi-Ling Yang, Email: wt.ude.uycn.liam@gnayly. Corresponding author.† Both authors contributed as well. Received 2014 November 6; Revised 2017 September 5; Adopted 2017 September 8. As a loop diuretic, bumetanide blocks the co-transport of cationt-chloride, and this property may allow bumetanide to act as an anxiolytic modulation of GABAergic synaptic transmission in the CNS. Its potential for the treatment of anxiety disorders deserves further examination. In this study, we evaluated the possible involvement of the basolateral nucleus of the amygdala in the anxiolytic effect of bumetanide. Slices of the brain were prepared from Wistar rats. In this study, extracellular recordings, stereotax surgery, fear-intensifying surprise response, monitoring of physical activity and western blotting were used. Systemic administration of bumetanide (15.2 mg·kg−1, i.v.), 30 minutes before fear stabilization, significantly inhibits the acquisition of fear-amplifying surprising response. Phosphorelation of EQF in the basolateral yu core of the amygdala was reduced after bumetanide administration. In addition, bumetanide suprafusion (5 or 10 μM) reduced long-term potentiation in the amygdala in a dose-dependent manner. Intra-amygdala infusion of bumetanide, 15 minutes before fear also blocked the acquisition of fear-amplifying scare response. Finally, the possible effect of bumetanide on conditioned fear was eliminated by side-by-side control experiments. These results suggest that the basolateral nucleus of the amygdala plays a key role in the anxiolytic effects of bumetanide. are transmembrane proteins that mediate the transport of chloride ions through the cell membrane (Blaesse et al., 2009; Ko et al., 2014). There are two main types of CCC in which chloride is co-productive with sodium ions and/or potassium ions. They may be discriminated against by their sensitivity to CCC inhibitors, transported ionic schiometry and phylogenesis (Payne and Forbush, 1995; Isenring and Forbush, 2001; Hannaert et al., 2002). Members of the sodium-bound branch, which consists of co-fixers Na-K-Cl, and move chloride ions into the cell and increase the intracellular chloride concentration ([Cl−]i) above the electrochemical balance of chloride. The potassium-bound branch, consisting of four different (and primarily transports chloride ions from the cell, reducing [Cl−]even under the electrochemical balance of chloride (Mount et al., 1998; Kahle and Staley, 2008, 2012; Dzhala et al., 2010). In adult neurons, [Cl−] is also lower than the level of extracellular chloride, and the reverse potential of chloride currents is close to the resting membrane potential of neurons. Even slight changes in [Cl−] i can significantly change the size, and even polarity, of GABA-mediated chloride current (Fiumelli and Woodin, 2007; Kahle and Staley, 2008, 2012; Ko et al., 2014). Anxiolytics such as benzodiazepines modulate GABAergic neurotransmission. Therefore, loop diuretics including furosemide and bumetanide are thought to have anxiolytic effects and have been tested in rodent models of both cue and contextual fear air conditioning (Krystal et al., 2012). The results showed that bumetanide significantly weakens both types of conditioned fear, but the detailed mechanism (mechanisms) has not been clarified. There is a significant amount of evidence to suggest that the basolateral number of the amygdala (BLA) is fundamentally involved in (LeDoux et al., 1990; Kim et al., 1991; Campeau and Davis, 1995; Fanselow and LeDoux, 1999; Maren, 1999). Either neurotoxic lesions or intracranial injections of NMDA receptor glutamate antagonists into bla blocked the acquisition and expression of conditioned fear (Miserendino et al., 1990; Kim et al., 1991; Maren et al., 1996; Gewirtz and Davis, 1997; Walker et al., 2002; Yang and Lu, 2005). Bla function is believed to be the basis of the neuroplasticity process, which pairs conditional stimulus (CS) and unconditional stimulus (US) - induced by afferent sensory and painful information (Fanselow and LeDoux, 1999; Yang and Lu, 2005). Although BLA's involvement in fear learning is widely accepted, the behavioral effects of centrally given bumetanide on acquiring conditioned fear remain unclear. Anxiety disorders are the most wide-used class of psychiatric conditions. A number of pharmacological substances are currently used as anxiolytics, including selective and submit=Search+Database. However, approximately 20 to 40% of patients reacted poorly to all available drugs (Denys and de Geus, 2005; Krystal et al., 2012). In addition, many of these anxiolytic substances can cause serious side effects (Hirschfeld, 2003; Denys a de Geus, 2005; Krystal et al., 2012). There is an urgent need for new anxiolytics, with fewer side effects and higher efficiency. The possibility of using loop diuretics as an anxiolytic deserves further investigation. The aim of this study was to evaluate the possible involvement of BLA in the anxiolytic effect of bumetanide. All animal care and experimental procedures have been in accordance with the Guide to National Institutes of Health for the Care and Use of Laboratory Animals and have been approved by the local institutional animal care; committee (IACUC) at National Taiwan Normal University. Adult male Wistar rats (purchased from BioLasco Taiwan Co., Ltd., Taipei, Taiwan) were used with a body weight of between 250 and 350 g. The animals were placed in group cages of four rats, each of which was in a temperature-controlled room, with free access to food and water. They were maintained in a cycle of 12:12 light-dark with lights at 0700 h. Animal studies are reported in accordance with arrive guidelines (Kilkenny et al., 2010; McGrath and Lilley, 2015). In this study, 124 rats were used and nine of them were excluded due to abnormal behavior or cannula error. Every effort was made to minimise the number of animals used, which was limited to the number needed to create meaningful experimental data. All behavioral experiments were conducted during the light cycle. Animals used for electrophysiological recording and western wandering were killed by cervical dislocation. They were then left for at least 5 minutes, confirming cardiac arrest and autonomic breathing before beheading. For systemic administration, (Sigma, St. Louis, MO) was freshly prepared with 0.5% saline with 0.5 N NaOH (O'Donnell et al., 2004) and then injected i.v. 30 minutes before training conditional fear. The selected dose (15.2 mg kg-1) was based on our previous experiments (Lu et al., 2006, 2008; Ko et al., 2014; 15.2 mg·corresponds to 42μmol). For extracellular recordings, bumetanide was first dissolved in 100% DMSO to form a stock solution of 10 mM and then diluted to 5 and 10 μM by artificial CSF (ACSF); composition: 117 mM NaCl, 4,7 mM KCl, 2,5 mM CaCl2, 1,2 mM MgCl2, 25 mM NaHCO3, 2 mM NaH2PO4 and 11 mM glucose; pH was maintained at 7.3-7.5 . The final DMSO concentration was approximately 0.05-0.10% (Kilb a.k.a., 2007). The intra-amygdala injection was performed through a 29-gauge injection of cannulas (model C313I, Plastic-one Products) and a Hamilton micro-syringe. The infusion rate and volume were 0.1 μL·min−1 and 0.3 μL per page. Rats were retained in a plexiglass cage, and a warm compress was applied for 2-3 minutes to provoke vasodilatation of the lateral causal veins. The tail was shaken with alcohol pads, and the needle of gauge 25 was inserted into a vein in a direction almost parallel to the vein. The application of negative pressure allowed a small amount of blood to enter the syringe and confirmed the correct location. The drug was gently injected into a vein, and the needle was removed from the vein. Pressure was applied to the puncture site with an alcohol pad until the rat stopped bleeding. Sodium pentobarbital (50 mg·kg−1, i.e.p.) has been used for animal anaesthesia. The animals were placed in a stereotaxic device (David Kopf instruments, Tujunga, CA, USA). Stainless steel guide cannulas (25 gauges, 12 mm) have been implanted into the blah or dorsal hippocampus (DH) bilaterally. Stereotaxic for the placement of guide cannulas in BLA and DH were front and rear (AP) = 2,8 mm, dorsal-ventral (DV) = 9,0 mm and laterally medial (LM) = +5,0 mm from bregmy; AP = 1.7 mm, DV = -1.5 mm and LM = +1.2 mm from bregma (Paxinos and Watson, 1997). Three steel screws and dental cement were used to anchor the guide cannulas. To prevent the cannula from clogging, stainless steel wires were inserted into each cannula. (100,000 IU·kg−1) was injected i.m. for 3 days to reduce the risk of infection (Corrigall et al., 1992). The animals were allowed to recover for 7 days prior to behavioural experiments. Rats were trained and tested in a commercialized pop-up system (San Diego Instruments, San Diego, CA, USA), and surprising responses were measured by moving the accelerometer under the blackmail chamber. The initial amplitude was defined as peak response within 200 ms after the onset of CS. The behavioural procedures included acclimatisation, the basic test phase, the fear stabilisation phase and the Fear Intensification Test (FPS), which are summarised below. Acclimatization was carried out on three consecutive days and the animals were placed in startled chambers for 10 minutes and then returned to their domestic cages. Neither CS nor the US were given to the animals. Basic surprise test In the next two consecutive days after the acclimatization phase, the animals were placed in startled chambers. 35 dB of surprising stimuli with an interstimulating interval of 30 s (ISI) were presented. Animals for which animals have been excluded compared to the average baseline of all animals compared to the average baseline have been excluded. Fear air conditioning Rats were exposed to fear air conditioning 24 hours later. They got 10 light leg-shock pairings. USA (foot shock) was delivered during the last 0.5 s 3.7 s CS presentation (light). The mean interval between the studies was 4 min (range = 3-5 min) and the shock intensity was 0.6 mA. Bumetanide was injected i.v. 30 min before the fear of air conditioning. A reaction to jumps caused by foot shock (shock activity) was noted. Fear-intensifying surprise test Rats were returned to the startled chambers 24 hours later and allowed to adapt for 5 minutes and before subsequent light noise tests were presented to them with 30 surprising noise discharges (95 dB, 30 s ISI) to obtain a stable base. A total of 60 surprising noise discharges were introduced. Thirty of them were in the dark (noise only) and 30 of them were presented 3.2 s after the onset of light 3.7 s (light noise). These surprising bursts of noise were presented in a balanced, irregular order with an ISI of 30 s, on average. The behavioral chamber is a cubic chamber of 48 cm each. Horizontal animal activity and stereotypical behaviour were monitored and recorded by commercial monitoring (Noldus Information Technology, Wageningen, Netherlands). Musculoskeletal monitoring was carried out immediately after the FPS test and the monitoring lasted 5 minutes. The coronal slices of the rat brain (400 μm thick) were prepared with a vibratom (MA752; Sigma, Campden Instruments Ltd, Loughborough, Leics, United Kingdom). The slices were placed in an acidated ACSF for at least 1 hour at room temperature to stabilize before recording. Each slice was then transferred to the recording chamber, where it was held submerged between two nylon nets and maintained at 32 ± 1 °C. ACSF was constantly perfused at a speed of 3-4 mL·min−1. Bipolar stimulating electrode (SNE-2OOX; David Kopf Instruments) was placed in a visible cell layer representing layer II/III neurons in BLA. Off-road EPSP (fEPSPs) were recorded extracellular using glass microelecterosis filled with 3 M NaCl (3-8 MΩ). Evoked fEPSP signals were recorded by Axoclamp-2B amplifier (Axon Instruments, Union City, CA, USA). Responses were low square pulses of 200 ms in duration, delivered at intervals of 20 s, filtered to 1 kHz and digitized to 5 kHz (Digidata 1322A; Axon Instruments). The stimulus voltage was individually adjusted for the production of fEPSP, which was 30-40% of the maximum response that could be triggered. The power of synaptic transmission was quantified by measuring the initial inclination of fEPSP and analyzed using pCLAMP software (version 10.2; Axon Instruments). Input-output (I/O) and joint facility (PPF) relationships were evaluated for measurement of basal synaptic transmission and presynaptic plasticity. I/O curves were obtained from eight incremental stimulation intensities (40-60 mV). Long-term potentiation (LTP) was triggered by high frequency stimulation (HFS) at an intensity twice as high as the test pulse. HFS consisted of two 1 with 100 Hz trains separated by an interlach interval of 20 s.Animals were killed by cervical dislocation 1 h after training. The animals were then decapitated and the amygdala was taken and briefly sonicated in an ice-cold homogenizing buffer. Protein concentrations were measured using the Bradford test, with BSA being the standard. The equivalent amount of protein for each sample was resolved in 10% SDS-PAGE gels and electrophoretically transferred to PVDF membranes and blocked overnight in 5% skim milk (Cell Signaling Technology, Inc., Danvers, MA, USA). The stains were incubated with antiphosphobic eq (New England Biolabs, Hitchin, UK) and antibodies to EQF (BD Biosciences, San Jose, CA, USA). Band detection was performed using the Western Blotting Analysis System (RPN 2108; Amersham International, Amersham, United Kingdom) (Yang and Lu, 2005; Ko et al., 2014). The data and statistical analysis are in line with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2015). The FPS test data were expressed as the average ± SEM and the average surprising sufficiency was analysed by ANOVA. comparisons were also carried out using bilateral t-tests for independent samples. Statistical analysis of LTP and PPF experiments was performed using the Mann-Whitney U-test. EQS phosphylation levels evaluated using western blot were analyzed using an unpaed student t-test. The materiality criterion for all comparisons was P < 0.05. All statistical analyses were performed using SPSS version 12.0 (SPSS, Chicago, IL, USA). The key protein targets and ligands in this article are hyperlinks to the corresponding entries in the , a common portal for data from the IUPHAR/BPS Pharmacology Manual (Harding et al., 2018) and are permanently archived in the Quick Pharmacology Guide 2017/18 (Alexander et al., 2017a, b, c). To confirm the previous findings Krystal et al. (2012) that systemic administration of bumetanide blocked conditional fear, 16 rats were randomly assigned to the control group and bumetanide-treated groups based on their surprising amplitum in the basal surprise test. The vehicle (control group) or bumetanide (15.2 mg·kg−1, i.v.) was injected 30 minutes before fear stabilisation (Figure 1A). The selected dose was based on our previous studies (Lu et al., 2006, 2008; Ko et al., 2014). The results showed that bumetanide treatment blocked the acquisition of FPS [t(1, 14) = 2,419] (Figure 1B). Immediately after the FPS test phase [t(1, 14) = −0.151] (Figure 1C) and physical activity (Figure 1D), no significant differences in shock activity were observed [t(1, 14) = −1,061]. Glutamate NMDA receptor and ERK signaling cascade in the amygdala play an important role in obtaining FPS (Gewirtz and Davis, 1997; Yang and Lu, 2005). To study the possible involvement of the EQF signaling pathway in the inhibition of FPS bumetanide, after stabilizing the fear, we evaluated the levels of EQF phosphylation in the amygdala after fear stabilization, western blotting. In short, 18 animals were randomly assigned to three groups, including the naïve control group, the group treated in the akumenz (control) and the bumetanide group. Groups treated with vehicles and bumetanide received one FPS training. The animals were killed 1 hour after training. EQF phosphylation in the amygdala in the vehicle group was significantly increased after fear stabilisation compared to the control group [t(1, 10) = 5,463] (Figure 2A, bars 1 and 2). This phospholylation of EQS was significantly reduced after bumetanide treatment [t(1, 10) = 6,856] (Figure 2A, stripes 2 and 3). The results of experiment 2 are summarized in Figure 2B. These results support our assumption that ERKs in the amygdala were involved in the effects of bumetanide on FPS. Our results showed that bumetanide blocked the acquisition of conditioned fear. The acute effect of bumetanide on the production of HFS-induced LTP in the amygdala was evaluated by an extracellular record. In mediated or different doses of bumetanide were administered 10 minutes before 10 minutes after stimulation. The results showed that a supraphusion of 10 μM bumetanide blocked the production of LTP, compared to groups of 5 μM bumetanide and in vehicle groups (vehicle group: 217 ± 14 %; 5 μM bumetanide group: 145 ± 12 % and 10 μM bumetanide group: 101 ± 4 %), n = 6 for each group (Figure 3A). In addition, there was no significant difference between animals in the vehicle group and animals treated with bumetanide as regards the I/O curve, indicating that the basic synaptic density and function were not affected by bumetanide (Figure 3B, left). Thus, the results obtained here would not be confounded by gross differences in synaptic organization or basic function. In addition, no significant differences were found in the PPF ratio between the groups (data were not shown), indicating that the presynaptic mechanism is also not involved in the blockade of LTP formation in the bumetanide-mediated amygdala. The results of experiment 1 showed that an I.V. injection of bumetanide interfered with the acquisition of FPS. After that, we assessed the role of the amygdala in the suppressive effect of bumetanide. The experimental procedure was summarised in Figure 4A. Initially, 20 bla-cannulated rats were used and five rats were excluded due to occlusion or incorrect location. The rats were then randomly assigned to two groups: the group treated with akut (ACSF infusion, n =7) or the bumetanide group (n=8) (Figure 4B). The vehicle or bumetanide was intracranially infused into bla 15 minutes before the fear of air conditioning. The animals were then tested for FPS 24 h later in the absence of drugs. The results showed that bumetanide blocked the acquisition of FPS compared to the vehicle group [t(1, 13) = 2.141] (Figure 4C). There were no significant differences in shock or physical activity between the groups (no data were shown). Campeau and Davis (1995) reported

that bla lesions blocked the expression FPS. Thus, the blockade of the acquisition observed during the test phase could result from the toxic action of bumetanide rather than from acute suppression in the acquisition. For toxicity testing, all animals were retrained and retested for FPS, 24 hours later in the absence of medication. The result showed that the animals in the bumetanide group showed significant FPS in the repeated test compared to the initial FPS test (Figure 4C, D) [t(7) = 3,041]. Thus, the inhibitory effect of bumetanide on FPS appeared to be a consequence of the acute effect of the drug rather than from any long-term lesion caused by bumetanide. Our experiments have shown that bumetanide blocked the acquisition of FPS. Since bumetanide was applied 15 minutes before fear air conditioning, it could affect both the acquisition and/or consolidation of FPS. In addition, it is possible that bumetanide has non-specific effects that block the acoustic response, which could be misinterpreted as a blocking effect on obtaining FPS. To exclude Option. Thirty-two animals were randomly included in two different sets (A and B), each of which consisted of two groups (n =8 per group). In set A, animals in the vehicle (control group) or bumetanide (in the bumetanide group) were immediately after fear adjustment to test the possible effect of bumetanide on consolidation. In kit B, the animals were subjected to intra-amygdala infension in the eggs or bumetanide (Figure 5A) before the test 15 minutes before the test. The results showed that intraamygdal infusion of bumetanide immediately after fear stabilisation showed a tendency to reduce FPS but did not achieve statistical significance [t(1, 14) = −0,217]. Compared to the intra-amygdala control group, fps expression was significantly blocked prior to the test [t(1, 14) = 4,531] (Figure 5C). These results indicated that the blockade of the SS is unlikely to be caused by the non-specific effect of bumetanide on consolidation. Previous studies have shown that systemic bumetanide blocked the contextual conditioning of fear and FPS. Then we wanted to check whether intra-hippocampal bumetanide influenced FPS. Initially, 17 cannula rats were used to test the possible involvement of the hippocampus, one of which is excluded due to occlusion. The animals were randomly classified in two groups, including the vehicle group (check) and bumetanide. The vehicle or bumetanide was infused into dh immediately after fear of air conditioning. The animals were subjected to FPS testing 24 hours later in the absence of drugs. The results showed that intra-hippocampal infusion of bumetanide had no effect on the acquisition of conditioned fear compared to the control group [t(1, 14) = −1.137] (Figure 6B). These results showed that the inhibitory effect of bumetanide on obtaining FPS did not include DH. Our results were consistent with previous results that acute bumetanide administration blocked the acquisition of FPS in rodents (experiment 1). In addition, we have shown that BLA plays an important role in the inhibitory effect of bumetanide. The possibility of a non-specific effect of bumetanide on basal surprise response or shock activity has been excluded. We also used western blot and in vitro extracellular recording to further determine a possible mechanism. In addition, our results showed that the level of PHOSPHORYlation of EQF in the amygdala was reduced after bumetanide administration (experiment 2). Finally, the size of HFS-induced LTP in the amygdala was suppressed depending on the dose after bumetanide supraphphis. These results are similar to our previous findings of the effects of bumetanide on hippocampal function (Ko et al., 2014). Taken together, these results suggest that BLA plays a key role in the anxiolytic effect of bumetanide. Krystal et al. (2012) reported that two diuretics, bumetanide and furosemide, exhibit anxiolytic effects in rats with fear-related models. They found that managing or furosemide significantly reduces both cue and contextual fear air conditioning. We have recently shown that bumetanide administration significantly weakens both the production of HFS-induced HIPOKAMPAL LTP and inhibitory avoidance learning in rats (Ko et al., 2014). Taken together, these results suggest that the CCC system may be involved in regulating anxiety and making it an important target for the development of new anxiolytics. Its clinical relevance for the treatment of anxiety disorders deserves further examination. In this study, we used a lower dose of bumetanide compared to the previous work krystal et al. (2012). The selected dose of bumetanide was based on our previous studies (Lu et al., 2006, 2008). After a dose of 50 or 70 mg·kg−1, we experienced non-specific effects including reduced basal response and decreased physical activity. Therefore, we used 15.2 mg·kg−1 for subsequent experiments. We suggested that the difference in strain (Long-Evans vs. Wistar rats) may represent the different doses that we used in this study. Bumetanide has been shown to affect NKC and KCC with different potency (Gagnon et al., 2007). It should be noted that KCC2 is also expressed in human CNS and that the dose chosen in this study (40 μM) is about 1/16 IC50 per KCC2 (Delpire a.k., 2009). Therefore, it is recommended that the effect observed here can be caused by the action only on the NKC in the brain. The permeation of bumetanide through the BBB has recently been demonstrated (Töllner et al., 2014; Donovan et al., 2015), how bumetanide exhibited anticonvulsant effects in both animal and human studies, suggesting that bumetanide was able to reasonably exceed the BBB (Sullivan et al., 1996; Lopez-Samblas et al., 1997; Wang and Kriegstein, 2011). In this study, in which we used foot shock as the US in the FPS paradigm, it is possible that nociceptive stimulus or fear itself may alter the function of BBB and lead to an increase in bumetanide entering the CNS. Due to poor penetration of BBB and rapid metabolism of bumetanide in rodents, it is necessary to assess the possibility of changing the function of BBB itself and increasing the penetration of bumetanide into the brain. According to our results, we cannot rule out the possibility that the acute effects of bumetanide on FPS have occurred with the secondary effect of bumetanide on metabolic, hormonal, cardiovascular, respiratory or other systemic mechanisms that are indirectly associated with the neuronal network. Since the supraphusion of bumetanide in brain incisions significantly suppressed the formation of LTP, it is unlikely that the acute effect would occur through the metabolite or secondary effect of bumetanide. In addition, the inhibitory effect of i.v. injection of bumetanide on FPS could be reproduced by infusion of intra-amygdala, which provides further evidence to support our hypothesis. One of the main issues derived from our results concerned the mechanism NKCC1 weakened eq phosphylation in the amygdala. Our previous studies have shown that the NKCC1 blockade reduced traumatic brain damage caused by neuronal damage and brain edema by reducing raf/MEK/ERK phosphylation (Lu et al., 2008; Ko et al., 2014). In neurons, phosphorylation of EQS can be increased by activating glutamate NMDA receptors (Atkins et al., 1998; Grewal et al., 1998) and results in an influx of calcium (Chetkovich et al., 1991; Chen et al., 1998). Therefore, bumetanide could reduce the activation of glutamate NMDA receptors in the amygdala after fear stabilisation and affect the subsequent pathway of signal transduction by direct effect on receptors or by reducing glutamate release (Su et al., 2002a,b). Another possibility is that the administration of bumetanide not only increased chloride gradients through the membrane of neurons, but also increased the amount of hyperpolarization after opening the chloride channel after activation of GABAA receptors (Dzhala et al., 2005; Blaesse et al., 2009), which is similar to the anxiolytic effect of benzodiazepines by increasing the influx of chloride by activating the GABAA receptor (Ravindran and Stein, 2010). However, blocking CCC with loop diuretics also affected intracellular ion concentrations and cell volume, which can significantly affect neuronal firing synchronization (Schwartzkroin et al., 1998; Hochman and Schwartzkroin, 2000: Haglund and Hochman, 2005; Ko et al., 2014). We would like to mention that we simply infused bumetanide into BLA and DH. We cannot rule out the possibility that other areas of the brain, such as the medial prefrontal cortex (mPFC), may be involved in the inhibitory effect of bumetanide on FPS. Further experiments, such as intracranial injections of bumetanide into mPFC, will be needed to test this option. Finally, we propose that the acute effect of systemic administration of bumetanide on FPS may be mediated by a central effect on BLA rather than effects on peripheral targets .M.-C.K. and M.-C.L. conducted experiments and analyzed the results. T.-H.T. conducted part of the electrophysiological experiments and analyzed the results. T.G.A. and M.A.T. participated in the design and discussion of behavioral study data. Y.-L.Y. and K.-T.L. designed studies, wrote a manuscript and provided all financial support. All the authors read and approved the final version of the manuscript. The authors do not declare any conflict of interest. This study was supported by the Ministry of Science and Technology, Taiwan, and this article was subsidized by the National Taiwan Normal University (NTNU), Taiwan, ROC. Ko, M.-C. , Lee, M.-C. , Tang, T.-H. , Amstislavskaya, T. G. , Tichonova, M. A. , Yang, Y.-L. and Lu, K.-T. (2018) Bumetanide blocks the acquisition of conditioned fear in adult rats. British Journal of Pharmacology, 175:1580-1589. Doi: [PMC free article] It's all right. [Google Scholar] Alexander SPH, Kelly E, Marrion NV, Peters JA, Faccenda E, Harding SD et al (2017a). Quick Guide to Pharmacology 2017/18: Transporters. Br J Pharmacol 174: S360-S446. [PMC free article] It's all right. [Google Scholar] Alexander SPH, Peters JA, Kelly E, Marrion NV, Faccenda E, Harding SD et al (2017b). Short Guide to Pharmacology 2017/18: Ligand-gated ion channels. Br J Pharmacol 174: S130-S159. [PMC free article] It's all right. [Google Scholar] Alexander SPH, Fabbro D, Kelly E, Marrion NV, Peters JA, Faccenda E et al (2017c). Quick Guide to Pharmacology 2017/18: Enzymes. Br J Pharmacol 174: S272-S359. [PMC free article] It's all right. [Google Scholar] Atkins CM, Selcher JC, Petraitis JJ, Trzaskos JM, Sweatt D (1998). The MAPK cascade is required for mammalian associative learning. Nature Neurosci 1: 602–609. It's all right. [Google Scholar] Blaesse P, Airaksinen MS, Rivera C, Kaila K (2009). Cotransports of cations and neuronal functions. Neuron 61: 820-838. It's all right. [Google Scholar] Campeau S, Davis M (1995). Involvement of the central nucleus and basolateral complex of the amygdala in fear stabilization as measured by fear-intensifying polishing in rats trained in parallel with auditory and visually conditioned stimuli. J Neurosci 15: 2301-2311. [PMC free article] That's fine. [Google Scholar] Chetkovich DM, Gray R, Jonhston D, Sweatt JD (1991). Activation of the N-methyl-d-aspartic receptor increases camp level and voltage-gated activity of the Ca2+ channel in the CA1 area of the hippocampus. Proc Natl Acad Sci USA 88: 6467-6471. [PMC free article] That's fine. [Google Scholar] Chen HJ, Rojas-Soto M, Oguni A, Kennedy MB (1998). Synaptic Ras-GTPAase-activating protein (p135 SynGAP) inhibited by CaM kinase II. Neuron 20: 895-904. It's all right. [Google Scholar] Corrigall WA, Franklin KB, Coen KM, Clarke PB (1992). The meso-imbic dopaminergic system is implicated in the strengthening effects of nicotine. Psychopharmacology 107: 285-289. It's all right. [Google Scholar] Curtis MJ, Bond RA, Spina D, Ahluwalia A, Alexander SP, Giembycz MA et al (2015). Experimental design and analysis and their reporting: new guidelines for publication in the BJP. Br J Pharmacol 172: 3461-3471. [PMC free article] That's fine. [Google Scholar] Denys D, de Geus F (2005). Predictors of pharmacotherapeutic response in anxiety disorders. Curr Psychiatry Rep 7: 252-257. It's all right. [Google Scholar] Delpire E, Days E, Lewis LM, Mi D, Kim KH, Lindsley CW et al (2009). The small molecule screen identifies k-cl kcc2 neuronal cotransport inhibitors. 106: 5383-5388. [PMC free article] That's fine. [Google Scholar] Donovan MD, Schellekens H, Boylan GB, Cryan JF, Griffin BT (2015). In vitro two-way throughput studies identify pharmacokinetic limitations of NKCC1 inhibitor bumetanide. Euros J Pharmacol 770: 117-125. It's all right. [Google Scholar] Dzhala VI, Kuchibhotla KV, Glycys JC, Kahle KT, Swiercz WB, Feng G et al (2010). accumulation of NKCC1-dependent neurononrides during Seizures. J Neurosci 30: 11745–11761. [PMC free article] That's fine. [Google Scholar] Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA et al (2005). The NKCC1 transporter facilitates seizures in the developing brain. Nature Honey 11: 1205-1213. It's all right. [Google Scholar] Fanselow MS, LeDoux JE (1999). Why do we think plasticity basic Pavlovian fear conditioning occurs in the basolateral amygdala. Neuron 23: 229-232. It's all right. [Google Scholar] Fiumelli H, Woodin MA (2007). The role of activity dependent regulation of homeostasis neuronalchloride in development. Curr Opin Neurobiol 17: 81-86. It's all right. [Google Scholar] Gagnon KB, Adragna NC, Fyffe RE, Lauf PK (2007). Characterization of glial cell K-Cl cotransport. Cell phyziol Biochem 20: 121-130. It's all right. [Google Scholar] Gewirtz JC, Davis M (1997). Second-order fear air conditioning prevent this by blocking NMDA receptors in the amygdala. Nature 388: 471-474. It's all right. [Google Scholar] Grewal SS, York RD, Stove PJ (1998). Extracellular signaling of kinase in neurons. Curr Opin Neurobiol 9: 544-553. It's all right. [Google Scholar] Haglund MM, Hochman DW (2005). Furosemide and mannitol suppress epileptic activity in the human brain. J Neurophyziol 94: 907-918. It's all right. [Google Scholar] Hannaert P, Alvarez-Guerra M, Pirot D, Nazareth C, Garay RP (2002). Rat NKCC2/NKCC1 cotransporter selectivity for loop diuretic drugs. Naunyn Schmiedebergs Arch Pharmacol 365: 193–199. It's all right. [Google Scholar] Harding SD, Sharman JL, Faccenda E, Southan C, Pawson AJ, Ireland S et al (2018). IUPHAR/BPS Pharmacology Guide 2018: updates and extensions to include a new immunopharmaceuticals guide. Nucl Acid Res 46: D1091-D1106. [PMC free article] It's all right. [Google Scholar] Hirschfeld RMA (2003). Long-term side effects of SSRIs: sexual dysfunction and weight gain. J. Clin Psychiatry 64 (Suppl 18): 20-24. It's all right. [Google Scholar] Hochman DW, Schwartzkroin PA (2000). The blockade of chloride-cotransport desynchronizes neuronal discharge in the epileptic hippocampal slice. J Neurophysiol 83: 406-417. It's all right. [Google Scholar] Isenring P, Forbush B (2001). Ionic transport and ligand binding cotransporter Na-K-Cl, study of structure function. Comp Biochem Physiol Mol Integr Physiol 130: 487-497. It's all right. [Google Scholar] Kahle KT, Staley KJ (2008). Cotransporter NKCC1 sensitive to Bumetanides Na-K-2Cl as a potential target of a new mechanism-based neonatal seizure treatment strategy. Neurosurgeon Focus. . It's all right. [Google Scholar] Kahle KT, Staley KJ (2012). Neonatal seizures and neuronal transmembrane ionic transport V: Jasper's basic mechanisms of epilepsy [Internet], 4. National Center for Biotechnology Information: USA. [Google Scholar] Kilb W, Sinning A, Luhmann HJ (2007). Model-specific effects of bumetanide on epileptiform activity in in-vitro in-vitro in-vitro in-vitro in-vitro in-vitro in-vitro in-vitro u novorozence myši. Neurofarmak 53: 524–533. To je v pořádku. [Google Scholar] Scholar] C, Browne W, Cuthill IC, Emerson M, Altman DG (2010). Animal research: in vivo experiment reporting: ARRIVE guidelines. Br J Pharmacol 160: 1577-1579. [PMC free article] That's fine. [Google Scholar] Kim JJ, DeCola JP, Landeria-Fernandez J, Fanselow MS (1991). Antagonist N-methyl-d-aspartic receptors APV blocks acquisition, but does not express fear. Behav Neurosci 105: 126-133. It's all right. [Google Scholar] Ko MC, Lee CL, Amstislavskaya TG, Tikhonova MA, Yang YL, Lu KT (2014). Inhibition of NKCC1 weakened hippocampal LTP formation and inhibitory avoidance in rats. PLos one. 9: e106692 . [PMC free article] It's all right. [Google Scholar] Krystal D, Sutherland J, Hochman DW (2012). Loop diuretics have anxiolytic effects on rat models of conditioned anxiety. PLoS One 7: e35417 . [PMC free article] It's all right. [Google Scholar] LeDoux JE, Cicchetti P, Xagoraris A, Romanski LM (1990). Lateral amygdaloid nucleus: sensory interface of the amygdala in conditions of fear. J Neurosci 10: 1062-1069. [PMC free article] That's fine. [Google Scholar] Lopez-Samblas AM, Adams JA, Goldberg RN, Modi MW (1997). Pharmacokinetics of bumetanide in a newborn. Biol Newborn 72: 265-272. It's all right. [Google Scholar] Lu KT, Wu CY, Cheng NC, Wo YYP, Yang JT, Yen HH et al (2006). Inhibition on the na+-k+-2cl−-cotransporter in the choroid plexus dampens traumatic brain injury caused by cerebral edema and neuronal damage. Euros J Pharmacol 548: 99-105. It's all right. [Google Scholar] Lu KT, Cheng NC, Wu Cy, Yang YL (2008). NKCC1-mediated traumatic brain injury-induced brain edema and neuron death through raf/mek/mapk cascades. Crit Care Med 36: 917-922. It's all right. [Google Scholar] Maren S (1999). Neurotoxic basolateral amygdala lesions disrupt learning and memory, but not the exercise of conditioned fear in rats. J Neurosci 19: 8696–8703. [PMC free article] That's fine. [Google Scholar] Maren S, Aharonow G, Fanselow MS (1996). Retrograde cancellation of conditioned fear after excitotoxic lesions in basolateral amygdala in rats: absence of temporal gradient. Behav Neurosci 110: 718–726. It's all right. [Google Scholar] McGrath JC, Lilley E (2015). Implementing guidelines for reporting on research using animals (ARRIVE, etc.): new disclosure requirements in the BJP. Br J Pharmacol 172: 3189-3193. [PMC free article] That's fine. [Google Scholar] Miserendino MJD, Sananes CB, Melia KR, Davis M (1990). Blocking the acquisition, but not expressing the conditioned fear amplified to polish nmda antagonists in the amygdala. Nature 345: 716-718. It's all right. [Google Scholar] Mount DB, Delpire E, Gamba G, Hall AE, Poch E, Hoover RS et al (1998). Electroneutral cotransports of cations and chlorides. J Exp Biol 201: 2091-2102. It's all right. [Google Scholar] O'Donnell ME, Train L, Lam TI, Liu XB, Anderson SE (2004). Bumetanide inhibition of hemoence-brain barrier Na-K-Cl cotransporter reduces production in the rat's middle brain occlusion model of stroke. J Cereb Blood Flow Metab 24: 1046-1056. It's all right. [Google Scholar] Paxinos G, Watson C (1997). Rat brain in stereotaxic coordinates. Academic press: New York. [Google Scholar] Payne JA, Forbush B (1995). Molecular characterization of epithelial isoforms of Na-K-Cl. Curr Opin Cell Biol 7: 493-503. It's all right. [Google Scholar] Ravindran LN, Stein MB (2010). Pharmacological treatment of anxiety disorders: an overview of progress. J Clin Psychiatry 71: 839–854. It's all right. [Google Scholar] Schwartzkroin PA, Baraban SC, Hochman DW (1998). Osmolarita, ionic flow, and changes in brain excitability. Epilepsy Res 32: 275-285. It's all right. [Google Scholar] Su G, Kintner DB, Flagella M, Shull GE, Sun D (2002a). Astrocytes of mice Na+-K+-Cl− cotransporter-null show the absence of swelling and decreased release of EAA. Am J Physiol 282: C1147-C1160. It's all right. [Google Scholar] Su G, Kintner DB, Sun D (2002b). Contribution of cotransporter Na+-K+-Cl− to high-vinegared swelling and release of EAA in astrocytes. Am J Physiol 282: C1136-C1146. It's all right. [Google Scholar] Sullivan JE, Witte MK, Yamashita TS, Myers CM, Blumer JL (1996). Evaluation of bumetanide pharmacodynamics in critically ill infants. Clin Pharmacol Ther 60: 424-434. It's all right. [Google Scholar] Töllner K, Brandt C, Töpfer M, Brunhofer G, Erker T, Gabriel M et al (2014). A new strategy based on prodruges to increase the effects of bumetanide on epilepsy. Ann Neurol 75: 550-562. It's all right. [Google Scholar] Walker DL, Ressler KJ, Lu KT, Davis M (2002). Facilitating the conditional extinction of fear by systemic administration or intra-amygdala infusions of d-cycloserin evaluated with fear amplified by the polisher in rats. J Neurosci 22: 2343-2351. [PMC free article] That's fine. [Google Scholar] Wang DD, Kriegstein AR (2011). Blocking early gaba depolarization with bumetanide results in permanent changes in cortical circuitry and sensorimotor gating deficits. Cereb Cortex 21: 574-587. [PMC free article] That's fine. [Google Scholar] Yang YL, Lu KT (2005). Facilitating the conditional extinction of fear by d-cycloserin is mediated by protein kinase-activated mitogen and PI-3 kinase cascades and requires the synthesis of de novo proteins in the basolateral nucleus of the amygdala. Neurosci 134: 247-260. It's all right. [Google Scholar] Articles from the British Journal of Pharmacology are provided here courtesy of the British Pharmacological Society