Implication of ATP-Sensitive K\(^+\) Channels in Various Stress-Induced Analgesia (SIA) in Mice

Kaoru Nakao, Masakatsu Takahashi and Hiroshi Kaneto

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Nagasaki University, 1-14, Bunkyo-machi, Nagasaki 852, Japan

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ABSTRACT—Exposure to footshock (2 mA, 1-sec duration, 0.2 Hz for 15 min; FS), forced swimming (water at 20°C for 3 min, SW) or psychological stress (using a communication box for 5 min, PSY) produced antinociceptive effects (stress-induced analgesia, SIA). Intracerebroventricular (i.c.v.) injection of glibenclamide (10–40 μg/mouse), an ATP-sensitive K\(^+\) (K\(_{\text{ATP}}\)) channel blocker, antagonized FS-SIA, while SW- and PSY-SIA were unaffected by the compound. Cromakalim (0.1–10 μg/mouse, i.c.v.), a K\(_{\text{ATP}}\)-channel opener, did not affect FS-, SW- or PSY-SIA. Thus, we provided evidence that central K\(_{\text{ATP}}\) channels participate in the production of FS-SIA but not production of SW- or PSY-SIA; and we suggest that glibenclamide, through closing of K\(_{\text{ATP}}\) channels, suppresses μ-opioid receptor functions, which subsequently leads to the inhibition of FS-SIA since antinociception is produced by the activation of μ-receptors.

Keywords: ATP-sensitive K\(^+\) channel, Stress-induced analgesia (SIA), Glibenclamide

Previous studies have indicated that glibenclamide, a potent sulfonylurea-derived blocker of ATP-sensitive K\(^+\) (K\(_{\text{ATP}}\)) channels, antagonizes the antinociceptive effects of morphine and the δ\(_{1}\)-opioid receptor agonist [\(\beta\)-Pen\(^2\),\(\beta\)-Pen\(^5\)]enkephalin (DPDPE), but not the δ\(_{2}\)-receptor agonist [\(\beta\)-Ala\(^{4}\)]deltorphin II or κ-receptor agonists U-69,593 and U-50,488H, in the mouse hot-plate (1) and tail-flick tests (2–4). Moreover, it has also been found that pinacidil and cromakalim, K\(_{\text{ATP}}\)-channel openers, potentiate the antinociceptive effects of morphine but not those of U-50,488H in the hot-plate test (5, 6). These results suggest that μ- and δ\(_{1}\)-opioid receptor agonists activate pain-inhibitory systems via opening of K\(_{\text{ATP}}\) channels.

We have previously shown that experimental animals exposed to footshock (FS), forced swimming (SW) or psychological (PSY) stress demonstrate an antinociceptive effect known as stress-induced analgesia (SIA) (7, 8), which might be elicited by activation of intrinsic pain-inhibitory systems. Furthermore, because FS- and PSY-SIA but not SW-SIA are antagonized by naloxone, we also suggested that while opioid systems appear to be involved in the production of FS- and PSY-SIA, SW-SIA is mediated by non-opioid systems (7–9).

In view of these findings, the present study was performed to clarify the involvement of the opening of K\(_{\text{ATP}}\) channels in the production of FS-, SW- and PSY-SIA in the mouse tail-pinch test.

Male ddY strain mice weighing 18–20 g (Otsubo Exp. Animals, Nagasaki) were maintained at an ambient room temperature of 22 ± 1°C with food and water available ad libitum; and after reaching 22 ± 2 g, they were used in these experiments. All procedures in this study were approved by the University Animal Care and Use Committee.

Cromakalim (a gift from Kirin Brewery Co., Ltd., Takasaki) and glibenclamide (Sigma, St. Louis, MO, USA) were dissolved in 6% dimethyl sulfoxide and 3% Tween 80 in saline, respectively, and injected intracerebroventricularly (i.c.v.) according to the method of Haley and McCormick (10) 10 min before exposure to each opioid agonist or stress in a volume of 10 μl/animal.

Details of exposure to FS-, SW- and PSY-stress have been described elsewhere (7, 8). Briefly, mice were exposed to FS (2 mA, 1-sec duration, 0.2 Hz for 15 min), SW (water at 20°C for 3 min) or PSY (communication box for 5 min), followed by immediate assessment of the antinociception.

The antinociceptive effect was measured by the tail-pinch method using an artery clip adjusted to a pressure of about 600 g (cut-off time of 15 sec), every 5 min from
immediately after termination of stress exposure for a period of 15 min.

Results are expressed as means±S.E.M. Following analysis of variance for repeated measures of the overall data to assess statistical significance, differences between the individual mean values in different groups were analyzed by Dunnett's test. P<0.05 was considered to indicate a significant difference.

In accordance with our earlier reports, exposure to FS-, SW- or PSY-stress produced short-lasting antinociception. FS-SIA was significantly antagonized by injection of glibenclamide at 20 and 40 μg/mouse 10 min before stress exposure, but treatment with glibenclamide at doses of 10 to 40 μg/mouse failed to inhibit SW- and PSY-SIA (Fig. 1), while cromakalim (0.1–10 μg/mouse) had no effect on any of these SIAs (Fig. 2). The vehicles themselves did not affect these SIAs (Figs. 1 and 2).

It has been reported that glibenclamide specifically inhibits K<sub>ATP</sub> channels in the CNS (11) and cromakalim opens these channels (12). Also, glibenclamide has been demonstrated to lack affinity for opioid receptors (13). In addition, it has been suggested that central K<sub>ATP</sub> channels are involved in the production of antinociceptive effects through μ- and δ<sub>1</sub>-opioid receptors but not κ-receptors using the mouse hot-plate (1, 5, 6) and tail-flick tests (2–4). This result is supported by our unpublished data determined by the mouse tail-flick method that the antinociceptive effects of morphine and DPDPE, but not that of U-50,488H, were reduced by i.c.v. glibenclamide and enhanced by i.c.v. cromakalim.

Exposure to FS-, SW- and PSY-stress produces the antinociceptive effect (7, 8). In this report, we found that only FS-SIA but not SW- or PSY-SIA was attenuated by glibenclamide. These results suggest that K<sub>ATP</sub> channels are involved in the production of FS-SIA, and since in our previous reports, we demonstrated that FS-, SW- and PSY-SIA are mainly induced through μ-opioid receptor, non-opioid and κ-opioid receptor mechanisms, respectively (7–9), the suppression of FS-SIA by glibenclamide is consistent with previous observations that central K<sub>ATP</sub> channels participate in the antinociception induced by activation of μ-, δ<sub>1</sub>-opioid receptors. In contrast, since we have also suggested that not only κ- but partially μ-receptor mechanisms are involved in the production of PSY-SIA (8), the possibility that different types of μ-receptors in relation to coupling with K<sub>ATP</sub> channels par-
ticipate in the production of FS- and PSY-SIA could not be excluded. Actually, Ocana et al. (14) has reported that the KATP channels are involved in the morphine, buprenorphine and methadone-induced antinociception but not fentanyl or levorphanol antinociception, suggesting the existence of at least two different forms coupled or uncoupled to KATP channels.

Meanwhile, cromakalim was incapable of affecting FS-SIA. The mechanism underlying the reduction of FS-SIA by glibenclamide without potentiation by cromakalim remains uncertain. However, the results suggest that it can be assumed that exposure to FS-stress may cause KATP channels to open sufficiently to mask the openers effect, through the agonist properties of FS stress on the μ-receptors (9), resulting in opening of the channels (4).

Thus, we demonstrated here that central KATP channels participate in the antinociception induced by exposure to FS-stress, and our findings suggested that glibenclamide suppresses μ-opioid receptor functions through closure of KATP channels, which subsequently leads to the inhibition of FS-SIA since antinociception is produced by activation of μ-receptors.

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