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Pharmacological Interaction between Gabapentin and Glibenclamide in the Formalin Test in the Diabetic Rat

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Abstract

There is evidence that local peripheral administration of gabapentin produces antinociception through the activation of the ATP-sensitive K+-channel. However, this interaction has not been evaluated systemically, nor in diabetic rat. This work was undertaken to determine whether glibenclamide has any effect on the systemic antinociception induced by gabapentin. Inflammatory pain was induced by injection of formalin in diabetic rats. Reduction of flinching behavior was considered as antinociception. Systemic administration of gabapentin (10-56 mg/kg, *i.p.*) produced a dose-dependent antinociception in both phases of the formalin test. Also, glibenclamide (1-10 mg/kg, *s.c.*) blocked the gabapentin-induced antinociception. Given alone glibenclamide did not significantly modify formalin-induced nociception in diabetic rats. Our data suggest that gabapentin is able to reduce formalin-induced nociception in streptozotocin-injected rats. In addition, these data are consistent with gabapentin-mediated activation of ATP-sensitive -K^+ channels to produce systemic antinociception in the formalin test in diabetic rats.

Introduction

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces [1]. Hyperglycemia is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially nerves and blood vessels [2]. The treatment of chronic diabetes includes drugs to lower blood sugar and drugs for the treatment of complications [1,2]. The oral hypoglycemic drug glibenclamide is an ATP-sensitive K^+ channel blocker used to treat non-insulin dependent diabetes [1,2]. Antidepressants such as amitriptyline and anticonvulsants such as gabapentin are proposed for the treatment of diabetic peripheral neuropathy [1,2]. Therefore, it is not uncommon that diabetic patients receive glibenclamide and gabapentin concurrently. Interactions between different drugs may either increase or decrease the pharmacological or toxicological effects of each component. This work was undertaken to determine whether glibenclamide has any effect on the systemic antinociception induced by gabapentin.

Material and Methods

Animals: Male Wistar rats (aged 8-10 weeks; 200-240 g) from our own facilities had free access to food and drinking water before experiments. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals [3], and were approved by the Institutional Animal Care and Use Committee (UAEH).

Diabetes. Rats were treated with streptozotocin (STZ; 55 mg/kg; i.p.). After 14 days of STZ administration, rats with blood glucose levels ≥ 250 mg/dL were included.

Measurement of Antinociceptive Activity: Rats were placed in open Plexiglas observation chambers for 30 min; then they were removed for formalin administration. Fifty-µL of diluted formalin (0.5%) were injected subcutaneously (*s.c.*) into the dorsal surface of the right hind paw. Animals were then returned to the chambers and nocifensive behavior was observed immediately after formalin injection. Nocifensive behavior was quantified as the numbers of flinches of the injected paw during 1 min-period every 5 min up to 60 min after injection. Formalin-induced flinching behavior is biphasic. The initial acute phase (0-10 min) is followed by a relatively short quiescent period, which is then followed by a prolonged tonic response (15-60 min). At the end of the experiment the rats were sacrificed in a CO_2 chamber.

Study Design: Rats received appropriate vehicle (1000 µl) or increasing doses of gabapentin (10-56 mg/kg, i.p.) 60 min before formalin injection. In order to determine whether systemic antinociception was mediated by K^+-channels, the effect of systemic pretreatment (90 min before) with the ATP-sensitive-K^+-channel blocker glibenclamide (1-10 mg/kg, s.c.) on the antinociceptive effect induced by gabapentin was assessed. Drugs were injected in a volume of 1000 µl. Rats in all groups were observed regarding behavioral or motor function changes induced by the treatments. This was assessed, but not quantified, by testing the animals' ability to stand and walk in a normal posture.

Data Analysis and Statistics: All experimental results are given as the mean ± S.E.M. for 5-6 animals per group. Curves were constructed plotting the number of flinches as a function of time. The area under the number of flinches against time curves (AUC), an expression of the duration and intensity of the effect, was calculated by the trapezoidal rule. Reduction of number of flinches or AUC of both phases is reported. Analysis of variance (ANOVA),

followed by Tukey´s test was used to compare differences between treatments. Differences were considered to reach statistical significance when $p<0.05$.

Results and Discussion

Systemic administration of gabapentin (10-56 mg/kg) produced a dose-dependent antinociception in both phases of the formalin test in diabetic rats (Fig. 1). This result is in agreement with the gabapentin (50 mg/kg)-induced antinociceptive effects on mechanical hyperalgesia and allodynia in diabetic rats [4]. The systemic administration of glibenclamide, an ATP-sensitive K^+ channel inhibitor [5], was able to reduce the antinociceptive action of gabapentin, suggesting that this drug could activate these channels to produce its antinociceptive effect in diabetic rats (Fig. 2). These results agree with previous observations showing that glibenclamide reduced the decrease of K+-evoked [^{3}H] noradrenaline release induced by gabapentin in human neocortical slices [6]. Moreover, our group has observed that gabapentin may activate ATP-sensitive K^+ channels in order to produce part of its spinal antiallodynic effect in the Chung model of neuropathic pain [7] and to generate its peripheral antinociceptive effect in the rat 1% formalin test [8].

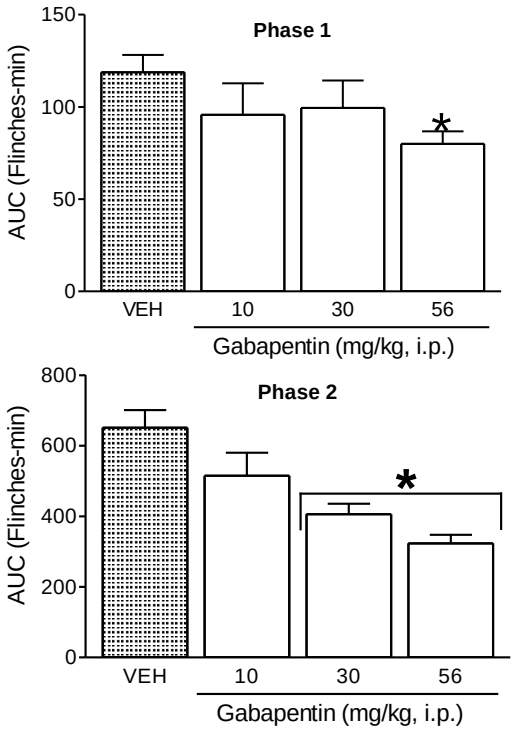

Figure 1. Systemic antinociceptive effect of gabapentin during the first and second phases of the formalin test. Diabetic rats received intraperitoneal pretreatment with vehicle or gabapentin and then formalin (0.5%) injection (50 µL). Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the mean ± S.E.M. for 5-6 animals. *Significantly different from vehicle group ($p<0.05$).

Administered alone, glibenclamide did not significantly modify formalin-induced nociception in diabetic rats. The lack of effect of this compound given alone is consistent with results of studies in which glibenclamide was not able to modify the nociceptive activity of formalin-induced nociception and mechanical hyperalgesia [9,10], thus excluding the possibility that the inhibition of gabapentin antinociception could be due to a hyperalgesic or nociceptive effect of the blocker.

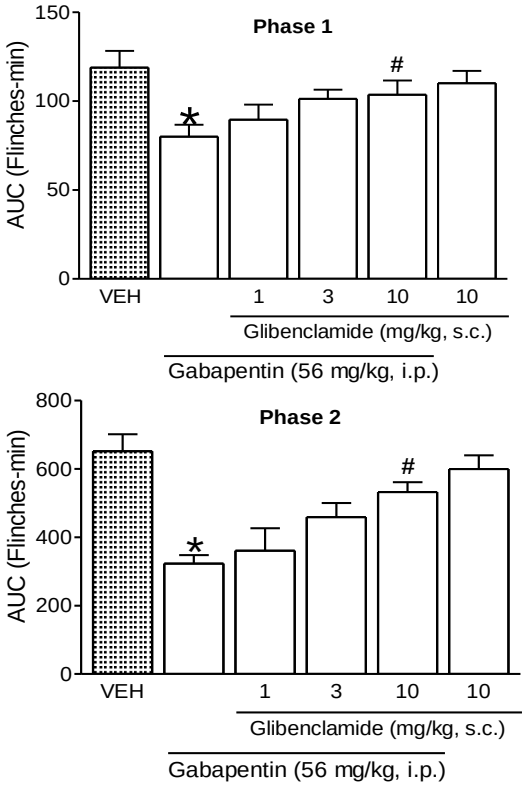

Figure 2. Effect of glibenclamide on the antinociceptive activity of gabapentin in the formalin test. Diabetic rats were pretreated with gabapentin (*i.p.*) plus glibenclamide (*s.c.*) before the formalin injection. Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the mean ± S.E.M. for 5-6 animals. *Significantly different from the vehicle group ($p<0.05$) and # significantly different from the gabapentin group ($p<0.05$).

Our data suggest that gabapentin is able to reduce formalin-induced nociception in streptozotocin-injected rats. In addition, data imply that gabapentin could activate the ATP-sensitive -K^+ channels in order to produce its systemic antinociceptive effect in the formalin test in diabetic rats. Likewise, data suggest that the gabapentin-glibenclamide combination can interact pharmacologically and therefore this drug association may represent a therapeutic dis-advantage in diabetic patients. Therefore, clinical studies assessing the possible interaction of this combination are needed.

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