# A Study of Glaucine-Induced Relaxation of Rat Aorta

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**Abstract**

The vasorelaxant effect of glaucine, the major alkaloid of *Platycapnos spicata* (L.) Bernh., was studied. At concentrations of 10 $\mu$M – 0.3 mM in normal Krebs solution it was almost equieffective in relaxing $K^+$-induced and noradrenaline-induced tension in rat aortic rings without endothelium, with $IC_{50}$ values of 160 ± 16 $\mu$M and 90 ± 14 $\mu$M respectively. In experiments in a calcium-free medium, 10 $\mu$M glaucine strongly inhibited noradrenaline-induced contractions. Glaucine (0.3 mM) did not affect basal uptake of $^{45}Ca$, but induced uptake was reduced to 100% ($K^+$) and 97.7% (noradrenaline) of the basal value. These results suggest that glaucine has an intracellular effect and also acts on the cell membrane by blocking voltage-dependent and receptor-operated calcium channels.

**Key words**

Glaucine, *Platycapnos spicata*, relaxing-effect, $^{45}Ca$ influx, rat aorta.

## Introduction

Many compounds with the aporphine skeleton are known to be pharmacologically active; examples include the dopaminergic agonist apomorphine and the dopaminergic antagonist bulbocapnine, which have both been extensively studied. It has been suggested that aporphine alkaloids and related substances, such as tetrahydropapaveroline, may be produced in the brain or other mammalian tissues, and that they may be involved in psychotic processes or functional disorders such as Parkinson's disease (1–3). The major alkaloid of *Platycapnos spicata* (4), glaucine, which also belongs to this family of compounds, is known to modify cerebral neurotransmitter and hormone levels (5, 6) and to relax smooth muscle, but little else is known of its effects and mechanism of action. In the work described here we investigated the mechanism by which glaucine isolated from *Platycapnos spicata* brought about the relaxation of isolated rat aorta, studying its effects on KCl-induced and noradrenaline-induced contractions, in normal and calcium-free medium, and on $^{45}Ca$ influx evoked by both vasoconstrictor agents.

## Materials and Methods

Male Sprague-Dawley rats weighing 250–300 g were killed by a blow on the head. The thoracic aorta was rapidly removed, stripped of endothelium by running a glass rod through the lumen, cut in cylindrical segments 4 mm in length and immediately transferred to an organ bath containing 20 ml of Krebs solution of the following composition (mM): NaCl, 118; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.2; $CaCl_2 \cdot 2H_2O$, 2.5; $KH_2PO_4$, 1.2; $NaHCO_3$, 25; glucose, 11. The solution was thermoregulated at 37 °C and bubbled with 95% $O_2$ + 5% $CO_2$.

Two stainless steel pins were introduced through the lumen of each arterial segment; one pin was fixed to the organ bath, and the other was connected to a CPOL force-displacement transducer for isometric tension recording by a computerized Celaster IOS 1 system.

After an equilibration period of at least 1 h under 2 g resting tension, isometric contraction forces induced by 10 $\mu$M noradrenaline or 60 mM $K^+$ (without keeping the osmolarity constant), were recorded over 15 minutes. Cumulative doses of glaucine were then added, and the effect of each one observed for 10 minutes.

To measure contraction forces in a calcium-free medium, artery preparations were equilibrated for 60 minutes in normal Krebs solution and then washed 3 times over a 20 minute period with Krebs solution containing 0.2 mM EGTA, instead of calcium, before a noradrenaline-induced contraction was elicited. To study the effects of glaucine, the preparations were immersed in normal Krebs solution for 60 minutes to replenish $Ca^{2+}$ stores depleted by the first contraction and then washed for 20 min in calcium-free solution before the desired concentration of glaucine was added, followed 10 minutes later by noradrenaline. Control preparations were simultaneously subjected to the same procedure but for addition of glaucine.

### *$^{45}Ca$ influx*

Aortic rings weighing 5–9 mg were equilibrated for at least 60 min in physiological solution composition (mM): NaCl 139, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1.5, HEPES 5, glucose 11, maintained at 37 °C and aerated with $O_2$. The tissues were then incubated for 5 min in a medium containing 0.6 $\mu$Ci/ml of $^{45}Ca$ (New England Nuclear, specific activity 35 mCi/mg) with or without 10 $\mu$M noradrenaline or 60 mM $K^+$, and with or without 0.3 mM glaucine. For incubation with glaucine, glaucine was also added to the equilibration bath 20 min before incubation with $^{45}Ca$.

Incubated preparations were washed for 5 min in 500 ml of $La^{3+}$ solution composition (mM): NaCl, 118; KCl, 5.9; Trishydroxymethylaminomethane, 5.4; $MgSO_4 \cdot 7H_2O$, 1.2; $LaCl_3 \cdot 7H_2O$, 50; glucose 11; pH 6.8. The arteries segments were then blotted, weighed and digested for 90 min in 1 ml of $H_2O_2$ (110 volumes) at 115 °C. After cooling, 5 ml of Beckman Ready-Safe was added and the radioactivity of the samples was measured in a liquid scintillation counter (Beckman LS 3801).

### *Calculation of characteristics and statistical analysis*

From the cumulative dose-response curves for the relaxant effects of glaucine, the 50% inhibition concentration ($IC_{50}$) was calculated.

The $^{45}Ca$ uptake was calculated as follows: $^{45}Ca$ uptake (nmol $^{45}Ca$/kg wet tissue) = [cpm in tissue/wet tissue weight (kg) × nmol $^{45}Ca$ in 1 litre solution/cpm in 1 litre solution]. Note that the numerator of the second factor in this expression is the concentration of $^{45}Ca$, not the total $Ca^{2+}$ concentration.

Unless otherwise specified, the results presented are means ± s.e.m. The statistical significance of differences between two means ($p < 0.05$) were estimated by Student's two tailed t test for paired or unpaired data.

### *Drugs and chemicals*

The following drugs were used: (+)-glaucine from *Platycapnos spicata* (4) and (−)-noradrenaline bitartrate (Sigma). Glaucine was dissolved in de-ionized water to make a 10 mM stock solution that was kept at − 20 °C. Appropiate volumes of this solution were taken and diluted immediately before use. Noradrenaline was prepared daily with de-ionized water from a 100 mM stock solution kept at − 20 °C and containing 0.2% sodium bisulphite to prevent oxidation. Chemicals used for physiological solutions were of analytical grade.

## Results

### *Vascular reactivity in normal Krebs solution*

The sustained contraction forces produced by noradrenaline and potassium in rat aortic rings reached maxima (100% response) of 2123 ± 236 for 10 μM noradrenaline and 2206 ± 185 mg for 60 mM $K^+$. Glaucine (10 μM − 0.3 mM) completely dose-dependently relaxed the tissue (Fig. 1) ($IC_{50}$ for noradrenaline = 90 ± 14 μM; $IC_{50}$ for $K^+$ = 160 ± 16 μM). The difference between these $IC_{50}$ values is not statistically significant at the $p > 0.05$ level (n = 7).

### *Vascular reactivity in calcium-free Krebs solution*

Noradrenaline (10 μM) produced a characteristic contraction with two distinct components: an initial transient contraction force of 1342 ± 104 mg (n = 6) that relaxed to a sustained force of 307 ± 18 mg. Both components were greatly reduced by 10 μM glaucine (Fig. 2), the fast component to 868 ± 113 mg and the slow component to 211 ± 25 mg ($p < 0.01$, n = 5).

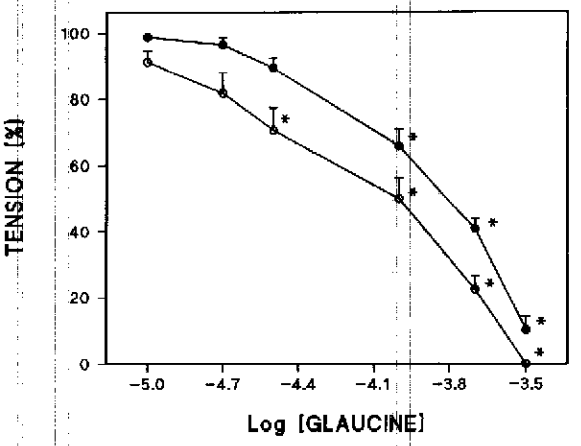


**Fig. 1** Effect of glaucine on potassium-induced (●) and noradrenaline induced (○) contractions of rat aortic rings. Points indicate means and bars s.e.m.'s of 7 rings. *$p < 0.05$ with respect to maximum tension.

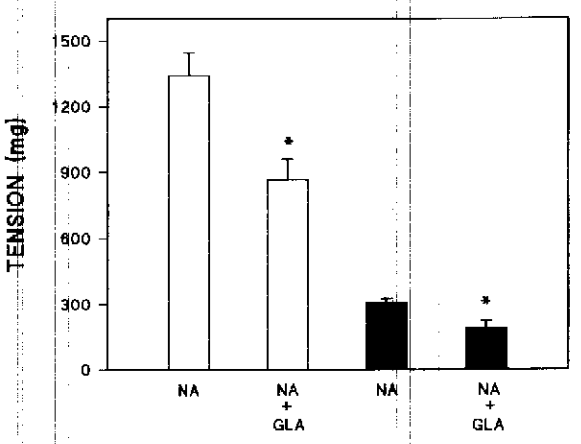


**Fig. 2** Transient (□) and sustained (■) contraction forces induced by noradrenaline in rat aortic rings, placed in a calcium free medium, in the presence or absence of 10 μM glaucine (GLA). Columns indicate means and bars s.e.m.'s of 5 rings. *$p < 0.05$ with respect to the value in the absence of GLA.

### *$^{45}Ca$ uptake*

The $^{45}Ca$ uptake by aorta segments in the absence of other agents (basal uptake) was 8.97 ± 0.39 nmol/kg (n = 10). Addition of glaucine (0.3 nM) had no significant effect (Fig. 3), increasing $^{45}Ca$ uptake to just 9.28 ± 0.29 nmol/kg (n = 6, $p > 0.05$).

Noradrenaline and $K^+$ significantly increased $^{45}Ca$ uptake to 12.21 ± 0.50 nmol/kg and 16.28 ± 0.63 nmol/kg, respectively, (n = 10, $p < 0.01$). Glaucine reduced noradrenaline and $K^+$-induced $^{45}Ca$ uptake to 9.14 ± 0.49 nmol/kg and 9.01 ± 0.64 nmol/kg, respectively (n = 6, $p < 0.01$).

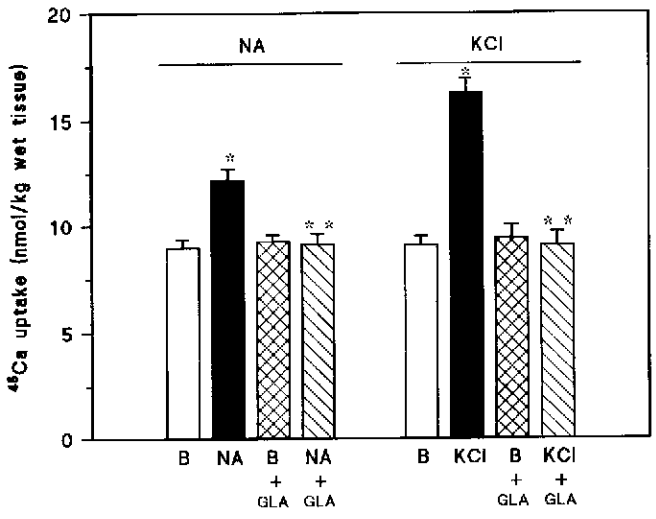


**Fig. 3** Effect of 0.3 mM glaucine (GLA) on basal, 10 $\mu$M noradrenaline-induced (NA) and 60 mM $K^+$-induced (KCl) $^{45}Ca$ influx in rat aortic rings without endothelium. Columns indicate means and bars s.e.m.'s of 6 rings. *$p < 0.05$ with respect to control (basal); **$p < 0.05$ with respect to $^{45}Ca$ uptake induced by NA or KCl.

## Discussion

The effect produced by glaucine, the main alkaloid of *Platycapnos spicata* (4), was studied on $K^+$-induced and noradrenaline-induced tension in rat aortic rings in standard and calcium-free medium, and on $^{45}Ca$ influx elicited by both vasoconstrictor agents. It has been reported that high K concentrations cause marked contractions in rat aortic tissue by depolarising smooth muscle cells and increasing the influx of calcium through L voltage-dependent channels (7–10). It has also been shown that activation of alpha$_1$-adrenergic receptors by noradrenaline in rat aorta induces a two-phase contraction: an initial fast transient contraction, caused by the $IP_3$-mediated release of calcium from intracellular stores, and a slow sustained contraction due to $Ca^{2+}$ influx through the receptor-operated $Ca^{2+}$ channels (11–14).

In this work, glaucine relaxed contractions induced by 60 mM $K^+$; this vasorelaxant effect is not due to its opening $K^+$ channels because cromakalim and other potassium channel openers do not relax contractions induced by $K^+$ concentrations greater than about 30 mM (15–16). The fact that the $IC_{50}$ values for $K^+$-induced and noradrenaline-induced contractions were almost equal suggests that glaucine acts intracellularly and/or on the cell membrane, blocking calcium influx through voltage-dependent and receptor-operated channels. The effects of glaucine within the cell are supported by the results obtained in a calcium-free medium, in which glaucine inhibited both the fast (transient) noradrenaline-induced contraction, attributed to calcium release from intracellular stores (via $IP_3$), and the subsequent sustained contraction that is thought possibly to involve the breakdown of phosphoinositide to diacylglycerol, activation of protein kinase C by the latter, and induction of contraction in the presence of a low concentration of $Ca^{2+}$ (17).

Also, glaucine may block calcium influx through the cell membrane; it had no effect on basal uptake of $^{45}Ca$, i.e. the amount entering the cell via leak channels (18), but greatly reduced uptake induced by $K^+$ and noradrenaline, suggesting that at the dosage used it blocks both voltage-dependent and receptor-operated Ca channels.

The mechanisms of intracellular action by glaucine are poorly understood, though it has been reported that at concentrations of 0.1–1.0 mM, glaucine increases intracellular cyclic nucleotide concentration in smooth muscle by inhibiting phosphodiesterase (19). Additional experimentation is in progress in order to clarify the precise mechanism of action of glaucine further.

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