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GW1229, a novel neuropeptide Y Y_1 receptor antagonist, inhibits the vasoconstrictor effect of neuropeptide Y in the hamster microcirculation

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Abstract

We studied the effect of GW1229, a novel neuropeptide Y Y_1 receptor antagonist, on the vasoconstriction induced by neuropeptide Y and structurally related analogs in the hamster cheek pouch microcirculation. Changes in arteriolar diameter and microvascular conductance were assessed by intravital microscopy and measurement of sodium^{22} clearance. GW1229 did not affect basal vascular conductance but inhibited, concentration dependently, the reduction in arteriolar diameter and vascular conductance induced by 100 nM neuropeptide Y. GW1229 also counteracted the vasoconstrictor effect of 100 nM [Leu^{31},Pro^{34}]neuropeptide Y, and that of 300 nM neuropeptide Y-(13–36). In contrast, GW1229 had no effect on the vasoconstriction induced by noradrenaline. We conclude that the vasoconstrictor effect of neuropeptide Y in the hamster cheek pouch is mediated by neuropeptide Y Y_1 receptors. The maintenance of physiological tone in this vascular bed does not involve the participation of endogenous neuropeptide Y.

Keywords: Neuropeptide Y receptor antagonist; GW1229; Neuropeptide Y Y_1 receptor; Microcirculation hamster

1. Introduction

Neuropeptide Y is a 36-amino-acid peptide which coexists with noradrenaline in most sympathetic nerves, including perivascular nerve terminals (Edvinsson et al., 1983; Ekblad et al., 1984). In the vascular system, application of exogenous neuropeptide Y elicits complex effects. In most vascular beds, neuropeptide Y potentiates the vasoconstriction caused by noradrenaline and other vasoactive agents (Edvinsson et al., 1984; Wahlestedt et al., 1985; López et al., 1989). In other vascular beds, neuropeptide Y decreases the release of noradrenaline from perivascular sympathetic nerve terminals (Coppes et al., 1994). Finally, in a few blood vessels, neuropeptide Y increases vascular resistance through a direct vasoconstrictor effect (Edvinsson et al., 1983; Mabe et al., 1985). This is the case for the hamster cheek pouch microvasculature, where topical application of neuropeptide Y has been recently shown to constrict both arterioles and venules (Kim et al., 1994; Boric et al., 1995).

Although neuropeptide Y has received much attention as a putative agent in the regulation of blood pressure, direct evaluation of the physiological role of endogenous neuropeptide Y has been precluded until recently by the lack of neuropeptide Y receptor antagonists with high affinity and specificity. Similarly, direct evidence for the participation of different neuropeptide Y receptor subtypes in the effects of exogenous neuropeptide Y has been hampered by the lack of potent antagonists with selective affinity for the different neuropeptide Y receptor subtypes.

Recently, four potent peptide antagonists for neuropeptide Y receptors have been reported (Daniels et al., 1995). Among them, compound GW1229 (see formula in Fig. 1) displayed high selectivity for neuropeptide Y Y_1 receptors, antagonizing with high potency both the neuropeptide Y-induced increase in perfusion pressure in the isolated rat kidney and the neuropeptide Y-induced increase in mean arterial blood pressure in anesthesized rats. Therefore, in this study we used this novel and apparently selective neuropeptide Y Y_1 receptor antagonist to determine whether the vasoconstrictor effect of neuropeptide Y in the hamster cheek pouch microcirculation is mediated by neuropeptide Y Y_1 receptors. In addition, we studied the effect of GW1229 on the maintenance of vessel diameter and vascular conductance in order to determine whether endogenous

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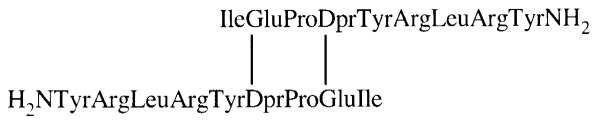

Fig. 1. Chemical structure of GW1229.

neuropeptide Y participates in the regulation of vascular tone in the hamster cheek pouch microcirculation.

2. Materials and methods

2.1. Microvascular flow and conductance determinations

Adult male golden Syrian hamsters (*Mesocricetus aureatus*) were anesthesized with 60 mg/kg sodium pentobarbital i.p. The trachea, left carotid artery and jugular vein were cannulated and the right cheek pouch was prepared for intravital microscopy as described (Boric et al., 1990, 1995). A lucite plate and a fiber optic bundle were introduced into the pouch for transillumination. The tissue was exposed, cleared of connective material, and a lucite observation chamber was placed and secured to the skin. The hamster was placed on the stage of a Nikon Optiphot microscope and the chamber was superfused with bicarbonate buffer (mM: 125 NaCl, 1.17 $MgSO_4$, 2 $CaCl_2$, 20 $NaHCO_3$), pH 7.4, 35°C, equilibrated with 95% N_2, 5% CO_2, at a flow of 1 ml/min. A glass cover-slide served to isolate the tissue from room air. Arterial pressure was registered with a Statham transducer and monitored on a Grass polygraph.

After a 45 min period of stabilization, 2×10^6 cpm of sodium^{22} in 0.2 ml was infused i.v. Another 20 min period was allowed for equilibration of the radioactive tracer between the plasma and the extracellular compartment. The experiment was started by collecting the cheek pouch superfusate output every 2.5 min. Duplicate 20 μl arterial blood samples were taken at the beginning and the end of the experiment. Radioactivity of the superfusate and plasma samples was measured in a LKB gamma counter, and clearance of the tracer was calculated as detailed by Boric et al. (1990). An index of relative vascular conductance was calculated at every time interval by dividing sodium^{22} clearance, assumed to represent absolute plasma flow of the superfused tissue, by the mean arterial pressure.

The microcirculatory network was examined with a 10× LWD Leitz objective and 10× Nikon oculars. A few selected fields of the cheek pouch microcirculation were video-recorded on a VHS tape at 10 min intervals, before, during and after exposure to the different concentrations of the vasoactive drugs. Vessel diameters were measured with a video caliper (Texas A&M University, College Station, TX, USA) during playback, at a magnification of 900×, with an accuracy of ±0.5 μm. Arterioles were classified according to their branching order, assuming the largest arteriole of the observed tissue to be of first order, and increasing one order each time a vessel divided into two of the same size. In the present study, A3 refers to arterioles between 16–30 μm; and A2 to arterioles of 31–60 μm in diameter, respectively.

2.2. Experimental protocols

All animals were monitored during a 30 min basal collection period. Thereafter the vasoactive drugs were applied directly to the superfusion buffer for periods of 20–30 min, and this was followed by a 60 min drug washout. Each animal was subjected to only one agonist or antagonist/agonist combined application. 4–5 hamsters were used in each group.

GW1229 was applied for 30 min at concentrations of either 100 or 300 nM. The agonists neuropeptide Y (100 nM), [Leu^{31},Pro^{34}]neuropeptide Y (100 nM), neuropeptide Y-(13–36) (300 nM) and noradrenaline (100 nM) were applied for 20 min, either alone, or after 10 min of superfusion with the neuropeptide Y antagonist. In the later groups, both the agonist and the antagonist remained in contact with the tissue for the next 20 min. The concentrations of neuropeptide Y, neuropeptide Y analogs and noradrenaline were chosen based on their vasoactive potency in the hamster cheek pouch microcirculation (Boric et al., 1995).

2.3. Quantification of results

Time courses of the relative conductance changes are shown as the means ± S.E.M. of the absolute values (nl/min per mmHg). Similarly, the time courses of changes in arteriolar diameters are presented in absolute values (μm). To compare the effect of the neuropeptide Y antagonist on the vasoconstriction elicited by different agonists, we measured the peak vasoconstrictor effect produced by the agonists in the presence and absence of GW1229, and expressed the relative vascular conductance as a standardized value relative to the averaged baseline (% decrease in relative vascular conductance).

The paired Student's *t*-test was used throughout to assess the significance of changes in relative vascular conductance and vessel diameter. Dunnet's tables for multiple comparisons with a single control were used when needed in time-course studies (Dunnet, 1964). In all cases, the level of statistical significance was set at a P value of < 0.05.

2.4. Animals and drug sources

The hamsters were bred at the University Animal Facilities; rodent's Purina chow and water were available at libitum. Neuropeptide Y, neuropeptide Y-(13–36), [Leu^{31},Pro^{34}]neuropeptide Y and GW1229 were synthe-

sized at Burroughs Wellcome Laboratories (Research Triangle Park, NC, USA) using solid phase methodology. The peptides were purified by high-performance liquid chromatography. Noradrenaline hydrochloride was from Sigma (St. Louis, MO, USA). Sodium^{22} (NEN, chloride, NEZ 081) was purchased from New England Nuclear (Boston, MA, USA).

3. Results

3.1. Antagonism of the vasoconstrictor effect of neuropeptide Y by GW1229

Neuropeptide Y (100 nM) reduced the relative vascular conductance, when applied topically to the hamster cheek pouch microcirculation (Fig. 2). This vasoconstrictor effect was marked and long-lasting and occurred in the absence of changes in systemic blood pressure. The change in vascular conductance was associated with a parallel decrease in the diameter of both A3 and A2 arterioles (Fig. 2).

Topical application of the neuropeptide Y receptor antagonist, GW1229, at a concentration of 0.1 or 0.3 μM for 30 min produced no significant changes in either the relative vascular conductance or the arteriolar diameter. Moreover, application of 1 or 10 μM GW1229 for 20 min produced no changes in arteriolar diameter (data not shown). However, GW1229 inhibited concentration dependently the reductions in relative vascular conductance and arteriolar diameter induced by 100 nM neuropeptide Y. While a partial inhibition was observed with 100 nM GW1229, a virtually complete inhibition of the vasoconstrictor effect of 100 nM neuropeptide Y was attained with 300 nM GW1229 (Fig. 2 and Fig. 3).

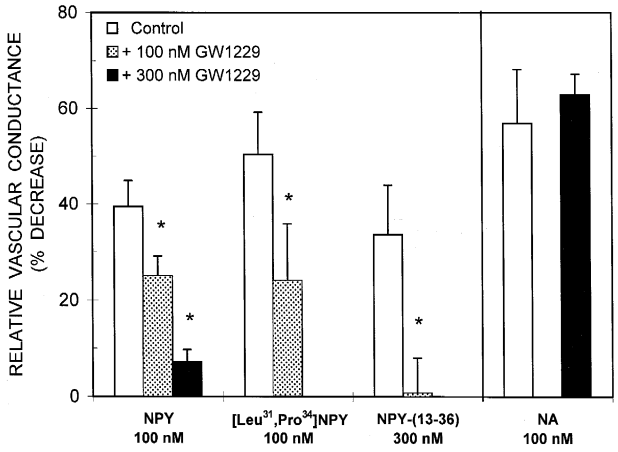

Fig. 3. Effect of GW1229 on the maximal drop in relative vascular conductance elicited by neuropeptide Y, [Leu^{31},Pro^{34}]neuropeptide Y, neuropeptide Y-(13–36) and noradrenaline (NA). Results are expressed as mean values ± S.E.M., obtained from 4–5 different hamsters. ★ $P <$ 0.05 relative to the corresponding values in the absence of GW1229.

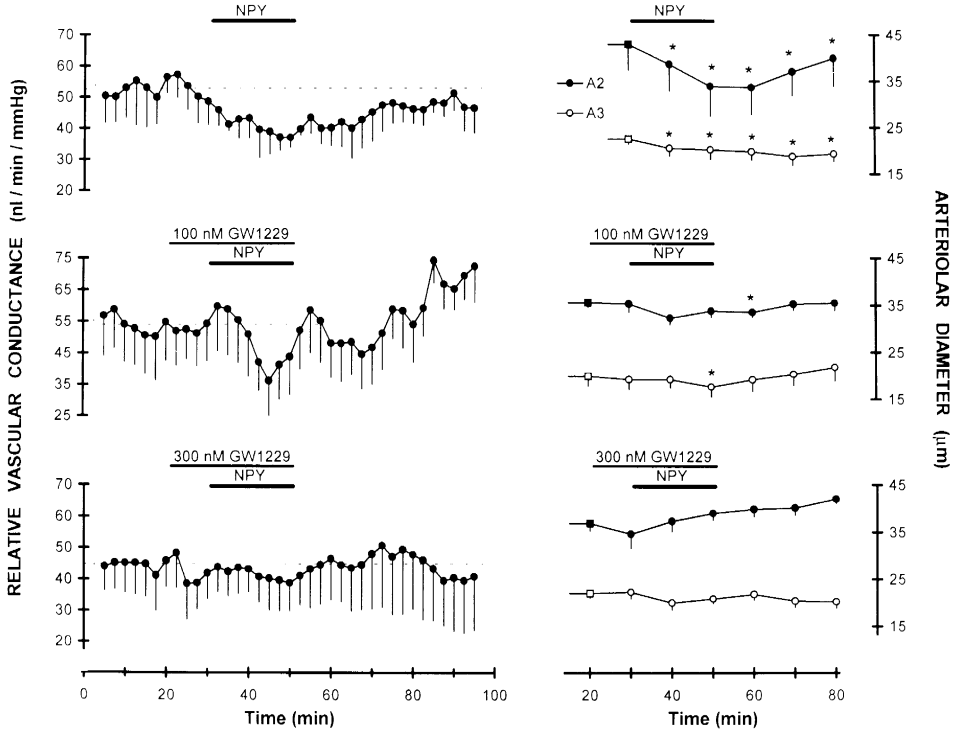

Fig. 2. Effect of GW1229 on the vasoconstrictor effect of 100 nM neuropeptide Y. Left panels show the time course of the changes in relative vascular conductance and right panels illustrate the time course of the changes in arteriolar diameter. In the later, filled symbols represent second-order arterioles (A2) and open symbols illustrate third-order arterioles (A3). Squares indicate the averaged baseline diameter of A2 and A3 arterioles prior to the addition of drugs. Results are expressed as mean values ± S.E.M. ($n = 4$–5). Lines above the tracings show the periods of application of both neuropeptide Y and GW1229. ★ $P < 0.05$ relative to the corresponding averaged baseline values.

3.2. Effect of GW1229 on the vasoconstrictor effects of [Leu[31],Pro[34]]neuropeptide Y, neuropeptide Y-(13–36) and noradrenaline

[Leu[31],Pro[34]]neuropeptide Y, a selective agonist for neuropeptide Y Y_1 receptors, reduced vascular conductance with a potency similar to neuropeptide Y (Fig. 3). In contrast, neuropeptide Y-(13–36), a neuropeptide Y Y_2 receptor-preferring agonist, displayed a much lower potency in this system (Fig. 3).

In addition to inhibiting neuropeptide Y-induced vasoconstriction, GW1229 antagonized the vasoconstrictor effect of [Leu[31],Pro[34]]neuropeptide Y, and that of a high concentration of neuropeptide Y-(13–36). In contrast, GW1229 had no effect on the vasoconstriction induced by 100 nM noradrenaline, when applied at concentration which effectively antagonized the effect of 100 nM neuropeptide Y (Fig. 3).

4. Discussion

The results of this study show that the novel neuropeptide Y receptor antagonist, GW1229, concentration dependently inhibited the vasoconstrictor effect of neuropeptide Y in the hamster cheek pouch microcirculation. In addition, GW1229 inhibited the vasoconstriction caused by the selective agonist of neuropeptide Y Y_1 receptors, [Leu[31],Pro[34]]neuropeptide Y. These results indicate that, as in other species (Daniels et al., 1995), the vasoconstrictor effect of neuropeptide Y in the hamster microcirculation is mediated by neuropeptide Y Y_1 receptors. In agreement with previous studies (Daniels et al., 1995; Hedge et al., 1995), our results show that the antagonism by GW1229 was selective for neuropeptide Y receptors, since the vasoconstriction induced by noradrenaline was not affected the neuropeptide Y receptor antagonist.

GW1229 behaves as a high-affinity and selective neuropeptide Y Y_1 receptor antagonist, both in vitro an in vivo. In radioligand binding studies, GW1229 displaces specifically bound ^{125}I-peptide YY from a human neuroblastoma cell line which is enriched in neuropeptide Y Y_1 receptors, with a potency 300-fold higher than that displayed in neuroblastoma cells that express neuropeptide Y Y_2 receptors (Matthews et al., 1995; Hedge et al., 1995). The low affinity of GW1229 for neuropeptide Y Y_2 receptors is confirmed by functional studies showing that in the vas deferens of the rat (neuropeptide Y Y_2 receptor assay), GW1229 has no effect on the neuropeptide Y-induced inhibition of both the electrically evoked twitch response (Matthews et al., 1995; Hedge et al., 1995) and the electrically evoked release of noradrenaline (Bitran et al., unpublished observations).

The observation that GW1229 virtually abolished the vasoconstriction induced by 300 nM neuropeptide Y-(13–36) argues in favor of the view that this neuropeptide Y fragment may non-selectively activate neuropeptide Y Y_1 receptors at this high concentration (Boric et al., 1995). In fact, when tested at lower concentrations, neuropeptide Y-(13–36) has no vasoconstrictor effect in the hamster cheek pouch microcirculation (Kim et al., 1994; Boric et al., 1995).

Besides having a direct vasoconstrictor effect, neuropeptide Y acts as a negative modulator of noradrenaline-induced vasoconstriction and as an enhancer of the hyperemia that ensues noradrenaline removal in the hamster cheek pouch microcirculation (Boric et al., 1995). Based on the use of selective agonists, these authors suggest that both neuropeptide Y Y_1 and Y_2 receptors are involved in these modulator effects of neuropeptide Y. More direct evidence for the participation of these neuropeptide Y receptor subtypes can now be obtained with the use of GW1229.

For many years the vasomotor effects of exogenously applied neuropeptide Y have been suggested to reflect a possible role of this peptide in the physiological regulation of blood pressure (Wahlestedt and Reis, 1993). Therefore, the availability of highly specific and potent neuropeptide Y receptor antagonists has been awaited with great expectation because of their potential to contribute to the unraveling of the physiological significance of endogenous neuropeptide Y in the control of blood pressure as well as other important functions. While GW1229 was shown to effectively inhibit the vasoconstrictor effect of neuropeptide Y in the isolated rat kidney as well as the neuropeptide Y-induced increase in peripheral resistance and increase in blood pressure in rats, this neuropeptide Y receptor antagonist failed to alter the resting mean arterial pressure in the rat (Daniels et al., 1995), suggesting that endogenous neuropeptide Y is not a major contributor to cardiovascular tone in anesthesized rats. In agreement with these findings, we observed no effect of GW1229 on the basal relative vascular conductance and resting diameter of vessels of the hamster cheek pouch microvasculature. The lack of effect of neuropeptide Y receptor blockade in this tissue was somehow expected since no detectable levels of endogenous neuropeptide Y are found in the hamster cheek pouch (Bitran et al., unpublished observations). Furthermore, it appears that sympathetic noradrenergic innervation is either minimal or non-existent in this tissue (Joyner et al., 1983).

In conclusion, the results of this study with the novel neuropeptide Y receptor antagonist, GW1229, give direct evidence that the vasoconstrictor effects of neuropeptide Y and structurally related peptides in the hamster cheek pouch microcirculation are mediated through the activation of specific neuropeptide Y Y_1 receptors. The maintenance of physiological tone in this vascular bed, however, seems not to involve the participation of endogenous neuropeptide Y.

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