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Short communication

# An antihypertensive opioid: Biphalin, a synthetic non-addictive enkephalin analog decreases blood pressure in spontaneously hypertensive rats

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ABSTRACT

*Background:* Endogenous opioid systems may be engaged in the control of arterial pressure (MAP), however, given the risk of addiction, opioid receptor agonists are not used in antihypertensive therapy. We examined cardiovascular effects of biphalin, a potentially non-addictive dimeric enkephalin analog, an agonist of opioid μ and δ receptors.

*Methods:* Biphalin was infused *iv* at 150 μg/kg/h to anesthetized spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). Along with MAP and heart rate (HR), renal blood flow (RBF) and iliac blood flow (IBF, a measure of hind limb perfusion) were measured using Transonic probes on renal and iliac artery, respectively. The effects of biphalin were compared with those of intravenous morphine (1.5 mg/kg/h).

*Results:* In two SHR groups biphalin decreased MAP from 143 ± 2 to 130 ± 2 and from 177 ± 4 to 167 ± 3 mmHg ($p < 0.001$) while HR did not change or modestly decreased. The renal blood flow (RBF) increased modestly and both renal and hind limb vascular resistances decreased significantly ($p < 0.001$). The responses were blocked by inhibition of peripheral opioid receptors with naloxone methiodide. Unlike in SHR, in WKY rats biphalin did not change MAP or vascular resistances. Morphine infusion decreased MAP in SHR from 169 ± 6 to 150 ± 6 mmHg (less decrease in WKY) and significantly decreased RBF and IBF.

*Conclusion:* Since biphalin, a non-addictive synthetic opioid, lowers MAP in SHR, a model of hypertension with pronounced neurogenic component, such analogs might find therapeutic application in human stress-induced hypertensive states. Biphalin's advantage is no associated reduction of renal perfusion.



## Introduction

Biphalin, first synthesized by Lipkowski et al. [1], is a dimer comprising two opioid tetrapeptide enkephalin analogs connected through a hydrazide bridge. It has almost equal affinity for μ and δ receptors [2]. Biphalin's affinity for μ receptor is similar to that of morphine; the observation that the analog's antinociceptive activity in animal models is much greater after intracerebroventicular administration [3] indicates its limited penetration of the blood–brain barrier [4].

Although the role of opioid systems in the control of cardiovascular functions has long been postulated and hypotensive action of morphine has been known for many decades [5,6], addictive properties of opiates precluded their application for chronic antihypertensive therapy. Considering biphalin's poor penetration of the blood brain barrier and the evidence from rat studies indicating that it displays only minimal physical dependence liability [7], the analog's addictive properties are very probably negligible. Therefore, biphalin or a compound with similar characteristics might have a therapeutic potential in hypertension, especially in the forms with pronounced sympathetic hyperactivity. This prompted us to examine effects of systemic (intravenous) administration of biphalin on blood pressure in spontaneously hypertensive rats (SHR), a model with a major neurogenic component [8,9]. Considering the uncertainty regarding the importance of cardiac *versus* peripheral vascular mechanisms in possible hypotensive action of opioids, we determined also the effect of biphalin on the resistance in renal and hind limb vascular beds.

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## Methods

Male spontaneously hypertensive rats (SHR, Polish Mother's Memorial Hospital Research Institute, Łódź, Poland) weighing 245–260 g, and male SHR and normotensive Wistar Kyoto rats (WKY) from another source (Animal House of the Medical University, Warszawa, Poland) weighing 270–310 g and 260–300 g, respectively, were used for experiments. They were anesthetized with intraperitoneal thiopentobarbital (Thiopental, Sandoz-Kundl, Austria), 100 mg/kg body weight. The experimental procedures were approved by the extramural IV Local Ethical Committee for Animal Experimentation, Warszawa.

We tested the effects on mean arterial pressure (MAP) and other circulatory parameters of biphalin, synthesized in house as described previously [1]. MAP and heart rate (HR) were measured *via* a femoral artery catheter connected with a pressure transducer and meter (Stoelting, Wood Dale, IL, USA). Saline and drugs were infused *via* a right femoral vein catheter. To measure hind limb perfusion (*i.e.* iliac blood flow, IBF), the area of aortic bifurcation was exposed from a suprapubic incision and a cuff Transonic probe was placed on the iliac artery and connected with a Transonic flowmeter (Type T106, Transonic System Inc., Ithaca, NY, USA). The left kidney was exposed from a subcostal flank incision and immobilized in a plastic holder. Another Transonic cuff probe was placed on the renal artery for measurement of total renal blood flow (RBF). The renal and iliac vascular resistances (RVR, IVR) were calculated as MAP-to-RBF ratio and expressed as mmHg/(ml/min). During surgery the rats received an *iv* infusion of 3% bovine serum albumin solution at 10 ml/kg/h. Thereafter, this infusion was replaced by isotonic saline and then drug solution in saline, given at the same volume infusion rate. The following experimental groups were studied:

*Biphalin studies*

(1) In SHR group from Łódź breeding center (SHR 1, $n = 8$, initial mean MAP = 143 ± 2 mmHg), after control (saline solvent) measurements, biphalin hydrochloride in saline was infused *iv* at 150 μg/kg/h during 30 min. Preliminary studies with doses between 100 and 300 μg/kg/h have shown that 150 μg/kg/h was the least dose which consistently decreased MAP in SHR. In this group the iliac blood flow (IBF) was not determined. In five experiments, after 20 min of biphalin infusion naloxone methiodide (Sigma, Basel, Switzerland), a blocker of peripheral opioid receptors, was injected *iv* at a dose of 200 μg/kg while biphalin infusion was continued. No post-biphalin recovery measurements were made in these experiments. We tested also that injection of naloxone alone did not change MBP or any hemodynamic parameters.
(2) In SHR from the Warszawa breeding center (SHR 2, $n = 18$, initial mean MAP = 177 ± 4 mmHg) the same protocol as in (1) was used except that post-biphalin recovery measurements were always obtained.
(3) The same protocol as in (2) was used in normotensive Wistar-Kyoto rats ($n = 10$)

*Morphine studies*

1. In SHR from the Warszawa breeding center the same protocol was applied but instead of biphalin, morphine (Polfa, Warszawa, Poland) in saline was infused *iv* at 1.5 mg/kg/h during 30 min ($n = 17$).
2. The same protocol was used in normotensive Wistar-Kyoto rats ($n = 9$).

**Statistical evaluation** employed the paired Student's $t$ test or repeated measurement ANOVA followed by a *post hoc* Tukey test. The standard error of mean (SEM) measured data dispersion, and $p \leq 0.05$ was accepted as significant.

## Results

Fig. 1 shows first an individual record of the response of MAP and RBF to a 30-min intravenous infusion of biphalin in SHR 1 group. MAP was seen to decrease within the first 4–8 min and remained lowered throughout infusion, with a clear recovery after its cessation. Simultaneously, RBF changed in the opposite direction. As seen from the mean values collected below the single record, biphalin decreased MAP from 143 ± 2 to 130 ± 2 mmHg while HR and RBF did not change. However, there was a modest but significant decrease in RVR. These results may suggest that the decrease in MAP was caused by a decrease in total peripheral vascular resistance rather than in the cardiac output.

The effects of biphalin were clearly reversed by naloxone (Fig. 2): during biphalin infusion MAP clearly decreased ($p < 0.01$) and then, after superimposed naloxone, it increased significantly ($p < 0.03$). Without background infusion of biphalin, naloxone did not alter any of the variables measured ($n = 11$, data not shown).

Effects of biphalin on MAP, HR, RBF, RVR, IBF and IVR in SHR 2 group and in normotensive WKY rats are shown in Fig. 3. In SHR, after biphalin infusion MAP decreased from 177 ± 4 to 167 ± 3 mmHg ($p < 0.001$) and after cessation of the infusion increased to 173 ± 3 mmHg, a value not significantly different from the pre-infusion control. The biphalin-induced MAP decrease was associated with a decrease in HR from 377 ± 7 to 354 ± 6 beats/min while RBF increased from 8.5 ± 0.5 to 9.3 ± 0.6 ml/min ($p = 0.001$) and RVR decreased significantly. IBF was not altered by biphalin while IVR decreased modestly but significantly. Unlike in SHR, in WKY rats neither of the parameters measured showed significant changes.

In SHR morphine induced a distinct progressing decrease in MAP: from control of 169 ± 6 to 150 ± 6 mmHg during infusion and further down to 138 ± 6 mmHg after its withdrawal ($p < 0.001$). A similar decreasing pattern but less steep and starting from normal baseline MAP level was also observed in WKY rats (Fig. 4). In both rat strains these changes were clearly dissociated from the concurrent minor oscillations in HR. RBF decreased progressively in SHR and WKY rats but the change was significant in the former only. Remarkably and unexpectedly, in SHR hind limb perfusion (IBF) increased and local vascular resistance (IVR) decreased from control of 43 ± 3 to 33 ± 3 mmHg/(ml/min) ($p < 0.004$), and remained lowered after withdrawal of morphine. This suggests a role of extrarenal vasodilation in the observed MAP decrease. In WKY rats neither of the parameters measured was affected by morphine infusion.

## Discussion

This is the first demonstration of a specific hypotensive effect of systemic administration of biphalin, a synthetic opioid, in hypertensive rats. The reversal of the post-biphalin pressure decrease with naloxone methiodide indicated that the effect was mediated by peripheral opioid receptors. The decrease in MAP in SHR was well reproducible, it was quite similar in two groups of rats derived from different breeding centers, the groups showing different baseline MAP. The biphalin-induced decrease in MAP was associated with a decrease in renal and hind limb vascular resistances but also in HR (seen in SHR 2 group only), which might suggest some reduction in the cardiac output. However, in a recent study loperamide, another μ-receptor agonist, did not alter the cardiac output in anesthetized SHR [10]. Collectively, these data suggest that the blood pressure lowering action of biphalin was