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Cannabinoid CB_1 receptor blockade enhances the protective effect of melanocortins in hemorrhagic shock in the rat

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Abstract

Activation of peripheral cannabinoid CB_1 receptors contributes to hemorrhagic hypotension, and endocannabinoids produced by macrophages and platelets may be mediators of this effect. A number of studies have provided evidence that functional links exist in the mechanisms of action of cannabinoids and opioid peptides; and opioids too play an important role in the pathophysiology of hemorrhagic hypotension and shock. On the other hand, melanocortin peptides, which are the main endogenous functional antagonists of opioid peptides, have an antishock effect in animals and humans. Thus, we investigated whether an interaction exists between endocannabinoids and the endogenous opioid/antiopioid system also in a condition of hemorrhagic shock and, particularly, whether the blockade of cannabinoid CB_1 receptors potentiates the antishock effect of melanocortins. Urethane-anesthetized rats were stepwise bled until mean arterial pressure decreased to, and stabilized at, 21–23 mm Hg. In this model of hemorrhagic shock, which caused the death of all control rats within 30 min after vehicle (tween 80, 5% in saline) injection, the intravenous (i.v.) bolus injection of the cannabinoid CB_1 receptor antagonist *N*-piperidino-5-[4-chlorophenyl]-1-[2,4 dichlorophenyl]-4-methyl-3-pyrazolecarboxamide (SR141716A) increased mean arterial pressure, pulse pressure, respiratory rate and survival rate in a dose-related manner (0.1–3 mg/kg), an almost complete recovery of mean arterial pressure, pulse pressure and respiratory rate, and 100% survival at the end of the observation period (2 h), occurring with the dose of 3 mg/kg. The melanocortin ACTH-(1–24) (adrenocorticotropin) also produced in a dose-related manner (0.02–0.16 mg/kg i.v.) a restoration of cardiovascular and respiratory functions, and increased survival rate, an almost complete recovery and 100% survival at the end of the observation period (2 h) occurring with the dose of 0.16 mg/kg. When a subactive dose of SR141716A (0.2 mg/kg; 30% survival) was associated with a subactive dose of ACTH-(1–24) (0.02 mg/kg; 12% survival), a complete reversal of the shock condition was obtained with 100% survival at the end of the 2-h observation period. The present results show that the concurrent inhibition of both endogenous opioid and cannabinoid systems produces a reversal of hemorrhagic shock more effective than that produced by the inhibition of either of them. These data suggest that functional interactions between endocannabinoids and opioid/antiopioid are at work also in the pathophysiology of hemorrhagic shock.

Keywords: Cannabinoid; Cannabinoid CB_1 receptor antagonist; Melanocortin; Hemorrhagic shock; (Rat)

1. Introduction

Cannabinoids have several pharmacological effects including vasorelaxant effects that are evoked through interaction with central or peripheral cannabinoid receptors (for reviews, see Randall and Kendall, 1998; Piomelli et al., 2000). So far, only a few investigations have been aimed at correlating endocannabinoid biosynthesis with particular pathophysiological conditions. Anandamide, or other acylethanolamides and *N*-acyl-phosphatidylethanolamines, for example, were shown to be synthesized following neuronal damage (Hansen et al., 1997), myocardial infarct and brain ischemia (for review, see Schmid et al., 1990). Cannabinoid CB_1 receptor agonists, in fact, increase neuronal survival in different animal models of brain ischemia (Nagayama et al., 1999) by activating presynaptic cannabinoid CB_1 receptors coupled to the inhibition of glutamate release (Shen and Thayer, 1998). Moreover, it has been reported that cannabinoid agonists modulate cytokines through cannabinoid CB_1 receptor activation in the inflammatory response to endotoxemia in mice (Smith et al., 2000); and Δ^9-tetrahydrocannabinol inhibits lypopolysaccharide-induced expres-

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sion of nitric oxide (NO) synthase through the inhibition of the transcriptional nuclear factor kB (NF-kB) (Jeon et al., 1996). In addition, cardioprotection against ischemia/reperfusion injury triggered by lypopolysaccharide involves endocannabinoids through activation of cannabinoid CB_2 receptors and could be attributed to a not yet identified relationship between endocannabinoids and NO (Lagneux and Lamontagne, 2001).

Recently, the overproduction of endocannabinoids has been correlated with hypotension in hemorrhagic and endotoxic shock in rats and humans (Wagner et al., 1997; Varga et al., 1998; Wang et al., 2001). Particularly, it has been suggested that both macrophage-derived anandamide and platelet-derived 2-arachidonyl glyceride may be mediators of such pathological conditions. Such circulatory derangement seems to be mediated by vascular cannabinoid CB_1 receptors because systemic, but not central, administration of the cannabinoid CB_1 receptor antagonist *N*-piperidino-5-[4-chlorophenyl]-1-[2,4 dichlorophenyl]-4-methyl-3-pyrazolecarboxamide (SR141716A) (Rinaldi-Carmona et al., 1994) reverses/prevents hypotension and increases survival rate (Wagner et al., 1997; Varga et al., 1998).

Besides endocannabinoids, it has been suggested that several endogenous compounds, such as NF-kB, tumor necrosis factor-α (TNF-α) and other cytokines, NO, oxygen-derived free radicals and platelet-activating factor (Hosford et al., 1989; Redl et al., 1993; Thiemermann et al., 1993; Altavilla et al., 1998, 2001; Borovikova et al., 2000) play an important role in the pathophysiology of circulatory shock.

Endogenous opioid peptides too play a role as mediators of circulatory shock. These peptides were recognized as early mediators of shock (Faden and Holaday, 1979; Schadt and Gaddis, 1985; Bertolini, 1995), and the opioid receptor antagonists naloxone and naltrexone reverse several shock states and increase survival rate in animals (Holaday and Faden, 1980; Gurll et al., 1982; Bernton et al., 1985).

A number of studies (for review, see Manzanares et al., 1999) have shown that opioids and cannabinoids share, besides antinociception, several pharmacological properties including sedation, hypothermia, hypotension, inhibition of both locomotor activity and intestinal motility; moreover, cross-tolerance or mutual potentiation of some pharmacological effects have been reported. These findings have supported the possibility that functional links exist in the mechanisms of action of both these groups of drugs.

We have previously shown that melanocortin peptides [adrenocorticotropin (ACTH), α-melanocyte-stimulating hormone (α-MSH) and other fragments lacking the C-terminal Arg–Phe sequence] have a life-saving effect in animals and humans in condition of hemorrhagic shock or splanchnic artery occlusion shock (Bertolini et al., 1986a,b,c, 1987; Bertolini, 1995; Squadrito et al., 1999; Noera et al., 2001). Melanocortins have a broad capacity to inhibit inflammatory processes and to regulate the immune system (Genedani et al., 1990; Lipton and Catania, 1997; Luger et al., 1997; Lipton et al., 1998; Wikberg, 1999; Catania et al., 2000); in particular, they reduce the production of pro-inflammatory cytokines, such as interleukin-1α, -1β, -6 and TNF-α (Luger et al., 1997; Lipton et al., 1998), while increasing the production of the anti-inflammatory suppressor factor interleukin-10 and angiogenic factor interleukin-8 (Luger et al., 1997; Wikberg, 1999). Also, the antishock effect of melanocortins is associated with a normalization of the blood levels of TNF-α, NO and oxygen-derived free radicals (Guarini et al., 1996, 1997; Altavilla et al., 1998).

Moreover, melanocortins are considered to be the main endogenous functional antagonists of opioid peptides (De Wied and Jolles, 1982; Bertolini et al., 1986d), and their antishock effect is antagonized by morphine (Bertolini et al., 1986b). Most likely, they activate or restore a complex vasomotor reflex that eventually leads to the mobilization of the peripherally pooled residual blood and which is seemingly obtunded by the massive release of endogenous opioids that occurs in such condition (for review, see Bertolini, 1995).

Thus, we aimed to investigate whether an interaction exists between endocannabinoids and the endogenous opioid/antiopiod system also in a condition of hemorrhagic shock and, particularly, whether the blockade of cannabinoid CB_1 receptors potentiates the antishock effect of melanocortins.

2. Methods

2.1. Animals and surgery

Wistar rats of both sexes (Harlan, Milan, Italy), weighing 270 to 300 g, were used. They were housed four per cage, males and females separately, with food in pellets and tap water freely available, in temperature (22 ± 1 °C)-, humidity (60%)- and ventilation-controlled colony rooms on a natural light/dark cycle. The animals were acclimatized to our housing conditions for at least 1 week before being used. The experiments were performed under urethane anesthesia (1.25 g/kg i.p.). Urethane (Fluka, Buchs, Switzerland) was chosen because it provides long-lasting and stable anesthesia with minimal interference with cardiovascular regulatory functions (Maggi and Meli, 1986). After heparinization (heparin sodium; 600 IU/kg i.v.), rats were instrumented with indwelling polyethylene catheters in a common carotid artery and an iliac vein. Systemic arterial pressure and pulse pressure were recorded by means of a pressure transducer (P23 Db; Statham, Osnard, CA) coupled to a polygraph (Mortara-Rangoni, Bologna, Italy). Respiratory rate was recorded by means of three electrodes subcutaneously implanted on the chest and connected to the polygraph through an ARI A380 preamplifier (Mortara-Rangoni).

Volume-controlled hemorrhagic shock was induced by stepwise bleeding from the venous catheter over a period of 25 to 30 min until mean arterial pressure, automatically

calculated by the polygraph, reduced to, and stabilized at, 22 to 25 mm Hg. The total bleeding volume was 2–2.5 ml/100 g b.w. The volume was similar for each experimental group, ranging from 2.15 ± 0.23 ($n = 8$) to 2.31 ± 0.18 ($n = 10$); $P > 0.05$, analysis of variance (ANOVA).

Arterial blood pressure and respiratory rate were recorded for 2 h after treatment, or until death if it occurred earlier.

All experimental procedures were carried out in accordance with guidelines of the European Community, local laws and policies (D.L. vol. 116/92).

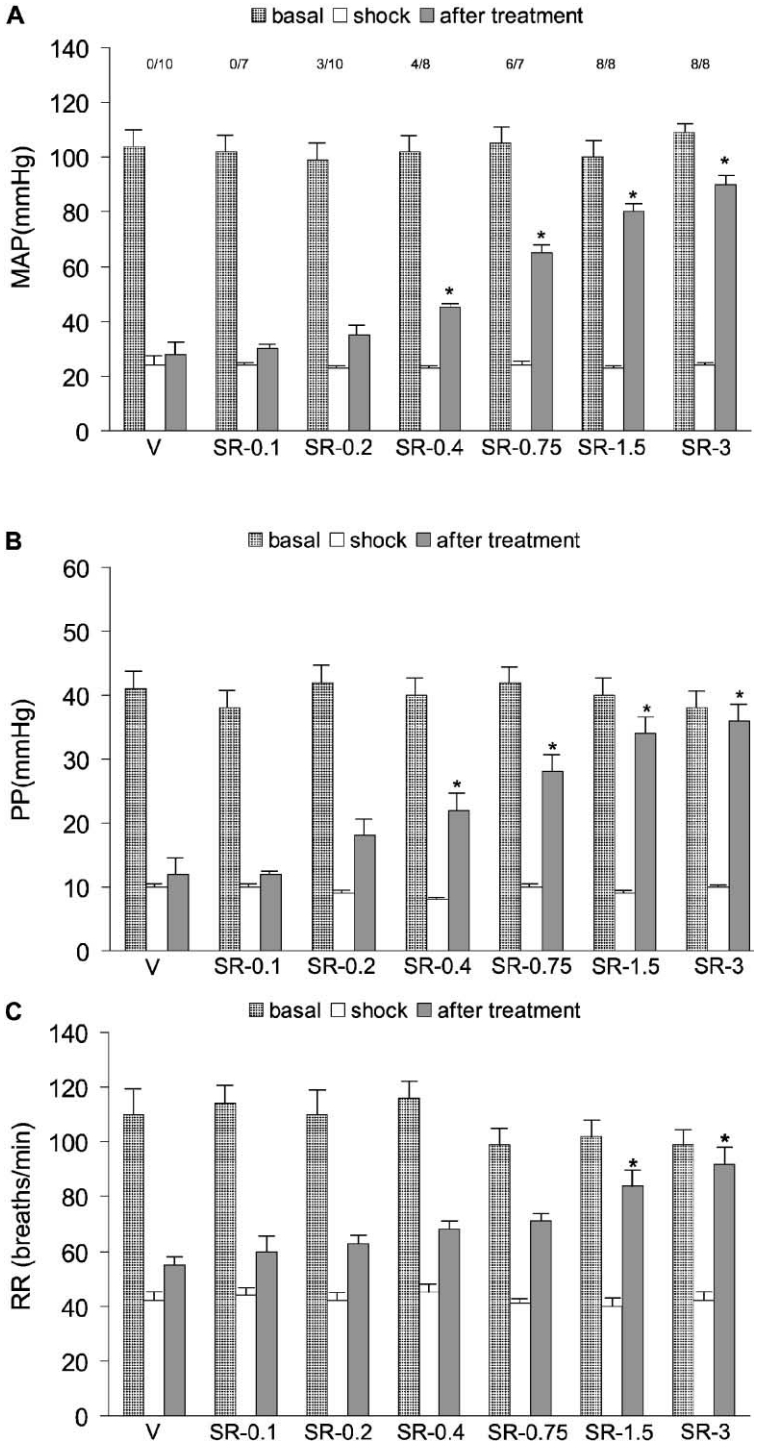

Fig. 1. Influence of i.v. treatment with SR141716A (SR, 0.1–3 mg/kg) on mean arterial pressure (MAP, panel A), pulse pressure (PP, panel B) and respiratory rate (RR, panel C) in hemorrhage-shocked rats. Histograms' height indicate mean values ± S.E.M. obtained before bleeding (basal), after bleeding (shock) and 10–15 min after treatment. V = vehicle, 1 ml/kg i.v. * $P < 0.05$, at least, vs. the corresponding value of V-treated rats (Student–Newmann–Keuls test). Comparisons between survival rates 2 h after treatment (figures above the histograms) gave significant P values starting from SR-0.4 ($P < 0.025$; Fisher's test).

2.2. Drugs and treatments

ACTH-(1–24), chosen as being the most effective melanocortin in the treatment of hemorrhagic shock (Bertolini et al., 1986c), and heparin sodium were purchased from Sigma (St. Louis, MO, USA); the selective cannabinoid CB_1 receptor antagonist SR141716A (Rinaldi-Carmona et al., 1994) was kindly provided by Sanofi Recherche (Montepellier, France). ACTH-(1–24) was freshly dissolved in saline shortly before use; SR141716A was freshly dissolved in tween 80 (0.17–5%) in saline. The i.v. injections were in a volume of 1 ml/kg 5 min after mean arterial pressure stabilization in the range of 22–25 mm Hg; control animals received equivolume amounts of saline or tween 80 in saline (vehicle).

2.3. Statistics

Mean arterial pressure, pulse pressure and respiratory rate values, and total bleeding volumes were analysed by means of ANOVA followed by Student–Newman–Keuls test. Survival rates were analysed by Fisher's exact probability test. A P value < 0.05 was considered significant.

3. Results

As repeatedly reported (Bertolini et al., 1986a,b,c; Guarini et al., 1996, 1997), the severe hypovolemia induced in our model of volume-controlled hemorrhagic shock in rats was incompatible with survival, and all vehicle-treated animals died within 30 min after treatment (Fig. 1).

The i.v. bolus injection, 5 min after bleeding termination, of the cannabinoid CB_1 receptor antagonist SR141716A increased, within 10–15 min, mean arterial pressure, pulse pressure and respiratory rate, and improved survival rate, in a dose-related manner (0.1–3 mg/kg), an almost complete recovery of cardiovascular and respiratory functions and 100% survival at the end of the 2-h observation period, occurring with the dose of 3 mg/kg (Fig. 1).

The melanocortin ACTH-(1–24), as repeatedly reported (Bertolini et al., 1986a,b,c; Guarini et al., 1996, 1997), also produced in a dose-related manner (0.02–0.16 mg/kg i.v.) and within 10–15 min, an almost complete and sustained restoration of mean arterial pressure, pulse pressure and respiratory rate and significantly increased survival rate (Fig. 2).

When a subactive dose of SR141716A (0.2 mg/kg), which produced a 30% survival, was associated with a subactive dose of ACTH-(1–24) (0.02 mg/kg), which produced a 12% survival, a reversal of the shock condition was obtained with an almost complete (within 10–15 min) and sustained restoration of mean arterial pressure, pulse pressure and respiratory rate and 100% survival at the end of the 2-h observation period (Fig. 3).

Neither vehicle (tween 80, 5% in saline i.v.), nor SR141716A (3 mg/kg i.v.) and ACTH-(1–24) (0.16 mg/kg i.v.) caused significant changes in arterial blood pressure

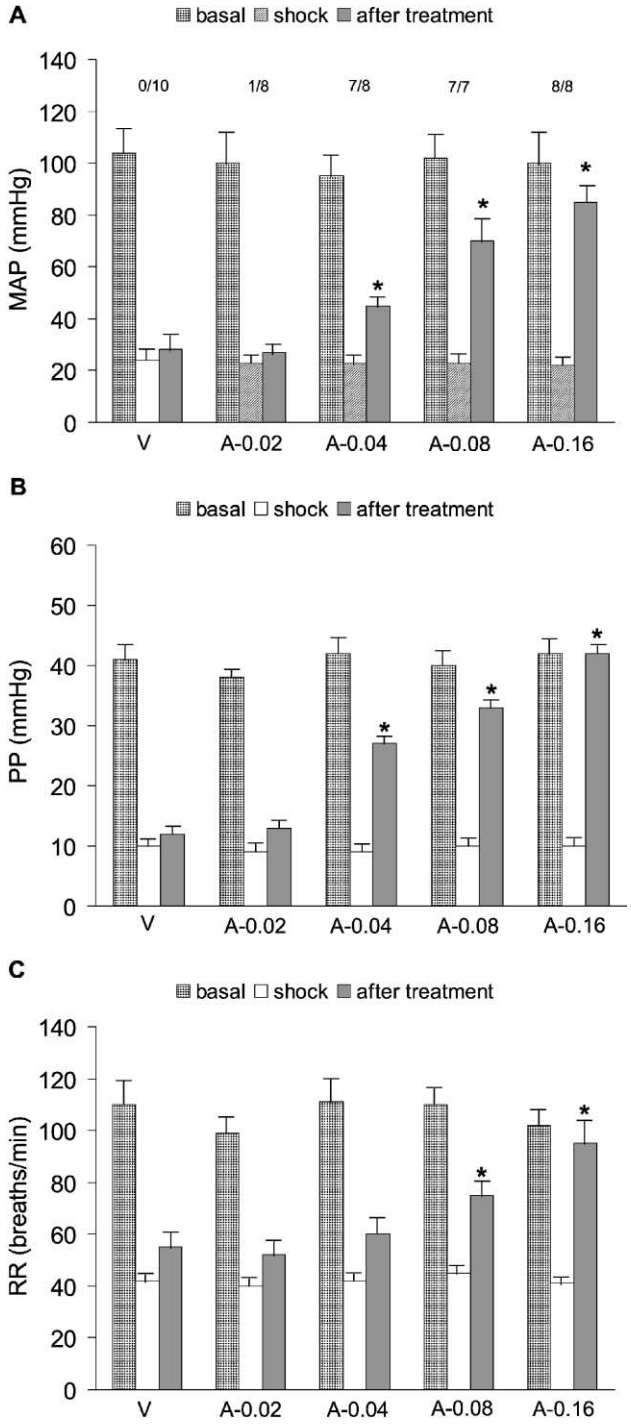

Fig. 2. Influence of i.v. treatment with ACTH-(1–24) (A, 0.02–0.16 mg/kg) on mean arterial pressure (MAP, panel A), pulse pressure (PP, panel B) and respiratory rate (RR, panel C) in hemorrhage-shocked rats. Histograms' height indicate mean values ± S.E.M. obtained before bleeding (basal), after bleeding (shock) and 10–15 min after treatment. V = vehicle, 1 ml/kg i.v. * $P < 0.05$, at least, vs. the corresponding value of V-treated rats (Student–Newmann–Keuls test). Comparisons between survival rates 2 h after treatment (figures above the histograms) gave significant P values starting from A-0.04 ($P < 0.005$; Fisher's test).

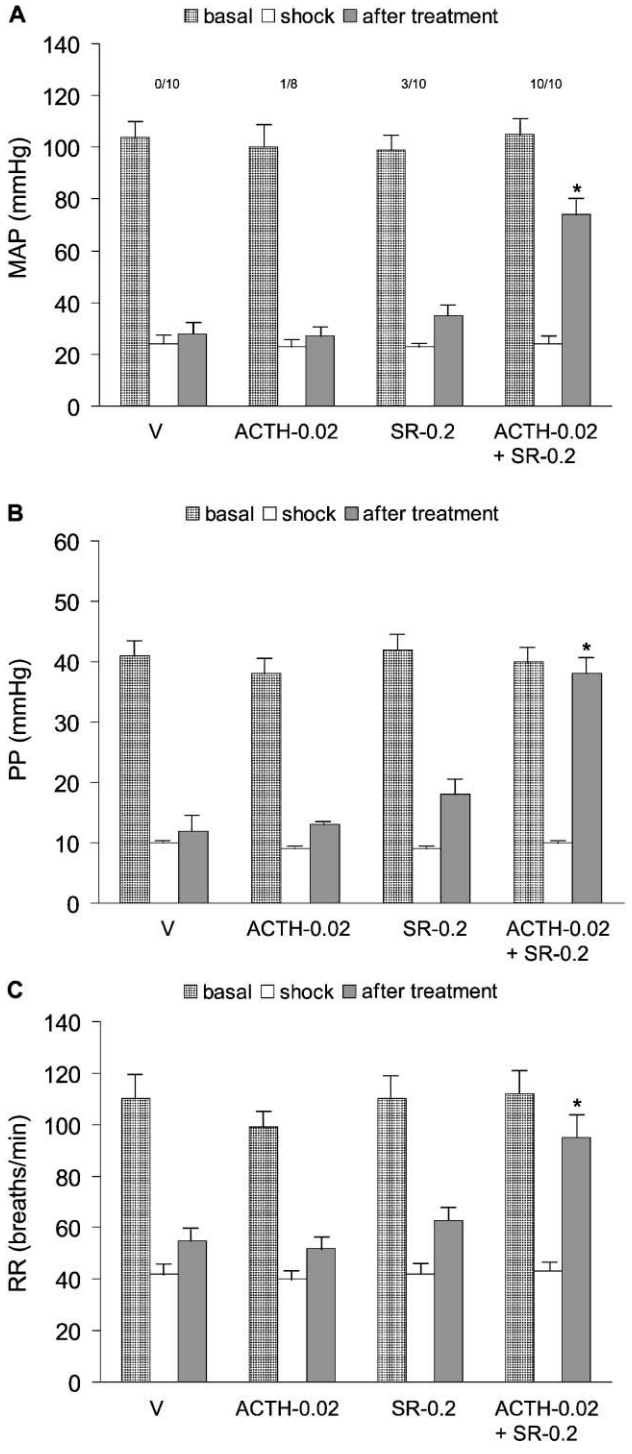

Fig. 3. Influence of i.v. treatment with association of subthreshold doses of ACTH-(1–24) (ACTH, 0.02 mg/kg) and SR141716A (SR, 0.2 mg/kg) on mean arterial pressure (MAP, panel A), pulse pressure (PP, panel B) and respiratory rate (RR, panel C) in hemorrhage-shocked rats. Histograms' height indicate mean values ± S.E.M. obtained before bleeding (basal), after bleeding (shock) and 10–15 min after treatment. V = vehicle, 1 ml/kg i.v. * $P < 0.05$, at least, vs. the corresponding value of V-treated rats (Student–Newmann–Keuls test). Comparisons between survival rates 2 h after treatment (figures above the histograms) gave significant P values only in the association-treated group ($P < 0.005$; Fisher's test).

and respiratory function when injected in normal, non-shocked rats (data not shown).

4. Discussion

Recent studies indicate that endocannabinoids are involved in the pathophysiology of circulatory shock (Wagner et al., 1997; Varga et al., 1998). Macrophage-derived anandamide and platelet-derived 2-arachidonyl glyceride seem to be among the mediators of such pathological condition. In fact, rat platelets and macrophages obtained from hemorrhage-shocked or lypopolysaccharide-treated rats, or exposed to lypopolysaccharide in vitro, trigger peripheral cannabinoid CB_1 receptor-mediated hypotension in normal recipient animals with decrease of survival. On the other hand, peripheral administration of the selective cannabinoid CB_1 receptor antagonist SR141716A (Rinaldi-Carmona et al., 1994) into rats bled to hemorrhagic shock causes a marked increase in blood pressure (Wagner et al., 1997). Shock condition may be also elicited by treatment with synthetic anandamide or 2-arachidonyl glyceride (Varga et al., 1998). High serum levels of anandamide and 2-arachidonyl glyceride have been detected also in human endotoxic shock (Wang et al., 2001).

Our data confirm the role of endocannabinoids in the pathophysiology of shock because the selective cannabinoid CB_1 receptor antagonist SR141716A, i.v. injected 5 min after bleeding termination, when mean arterial pressure is stabilized in the range of 22–25 mm Hg reverses the hemorrhage-induced shock condition.

On the other hand, as far as acute survival is concerned, our present data are at variance with those previously obtained by Wagner et al. (1997). Indeed, these authors found that i.v. pretreatment of rats with 3 mg/kg SR141716A before starting bleeding reduced the survival time, (whereas—as quoted before—treatment of rats after bleeding termination improved blood pressure) and inferred that activation of cannabinoid CB_1 receptors may be beneficial for survival in shock probably through a favourable redistribution of cardiac output or improved microcirculation by localized vasodilation. However, these same authors found that intraperitoneal treatment of rats with 3 mg/kg SR141716A 30 min before and 6 h after injection of E. coli endotoxin (lypopolysaccharide) significantly increased the survival from endotoxic shock (Varga et al., 1998). The disagreement between our data and part of the however rather variable data of Wagner et al. (1997) and Varga et al. (1998) may well depend on the different treatment schedules and on the different severity of the shock condition.

Moreover, the present data show that the blockade of cannabinoid CB_1 receptors enhances the hemorrhagic shock-reversing effect of the melanocortin ACTH-(1–24), a complete protection occurring with a dose of ACTH-(1–24) (0.02 mg/kg) eightfold lower when associated with a subactive dose (0.2 mg/kg) of SR141716A.

As repeatedly supported by sundry-independent experimental findings, melanocortin peptides are considered the main physiological antagonists of endogenous opioid peptides (Wiegant et al., 1977; Jacquet, 1978; Bertolini and Ferrari, 1982; De Wied and Jolles, 1982; Bertolini et al., 1986d). Opioids and melanocortins, in fact, exert opposite effects in many important body functions including cardiovascular function (Bertolini, 1995). Their antagonism may be due to an opposite effect on some neuronal functions (neuronal firing, transmitter release), or on second messengers (Ca^{2+}, cAMP), or both. Moreover, melanocortins and endorphins are synthesized and coreleased by the same cells and derive from a common macromolecular precursor (pro-opiomelanocortin) (Mains et al., 1977). We have suggested that, in shock conditions, melanocortins activate a vasomotor reflex that eventually leads to the mobilization of the peripherally pooled residual blood, the first step likely being the activation of melanocortin MC_4 receptors in the central nervous system (Guarini et al., 1999). This reflex may be obtunded by the well-known massive release of endogenous opioids that occurs in such conditions (for review, see Bertolini, 1995).

Other quite peculiar properties of melanocortins play a key role in their antishock effect: they greatly reduce the blood levels of oxygen-derived free radicals including NO, and of TNF-α that are massively increased in conditions of hemorrhagic shock (Guarini et al., 1996, 1997; Altavilla et al., 1998).

On the other hand, opioids and cannabinoids share several pharmacological effects such as antinociception, hypothermia, sedation, hypotension, inhibition of locomotor activity and intestinal motility (for review, see Manzanares et al., 1999), and a number of findings support the possibility that functional links exist in the mechanism of action of cannabinoids and opioids.

The precise nature of the cannabinoid–opioid interactions remains to be elucidated. It has been suggested that cannabinoids and opioids might interact at the level of signal-transduction mechanisms because their receptors are coupled to similar intracellular signalling mechanisms such as the decrease in cAMP production through activation of G_i proteins (Childers et al., 1992). This requires that receptors for both drugs be colocalized in the same cells, so to utilize the same pools of G proteins, but this is controversial (Shapira et al., 1998). However, several brain structures abound in both cannabinoid and opioid receptors (Mansour et al., 1988; Mailleux and Vanderhaeghen, 1992).

Alternatively, the cannabinoid–opioid interactions could be explained by the cannabinoid-induced increase in the synthesis and/or release of opioids (Manzanares et al., 1999).

In conclusion, the present data confirm the important role of endocannabinoids in the pathophysiology of shock and show that the concurrent inhibition of both endogenous opioid and cannabinoid systems produces a reversal of hemorrhagic shock more effective than that produced by the inhibition of either of them. These data suggest that functional interactions between endocannabinoids and the opioid/antiopioid endogenous system are at work also in the pathophysiology of hemorrhagic shock and that the combination of a melanocortin with a cannabinoid CB_1 receptor antagonist might be a more rational and effective treatment than either of them in the early critical care of this life-threatening condition.

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