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The Serotonin 5-HT1A-receptor agonist, 8-OH-DPAT, stimulates sympathetic-dependent increases in venous tone during hypovolemic shock

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Abbreviations:

8-OH-DPAT	(+) 8-hydroxy-2-(di-n-propylamino)-tetralin
MCFP	Mean Circulatory Filling Pressure
5-HT	5-hydroxytryptaphan
HR	Heart Rate
VPP	Venous Plateau Pressure
FAP	Final Arterial Pressure
ANOVA	Analysis of Variance
CVP	Central Venous Pressure
MAP	Mean Arterial Pressure

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Abstract

Adjuvant treatment of hypovolemic shock with vasoconstrictors is controversial due to their propensity to raise arterial resistance and exacerbate ischemia. A more advantageous therapeutic approach would utilize agents that also promote venoconstriction to augment perfusion pressure through increased venous return. Recent studies indicate that 5-HT_{1A}-receptor agonists increase blood pressure by stimulating sympathetic drive when administered after acute hypotensive hemorrhage. Given that venous tone is highly dependent upon sympathetic activation of α2-adrenerigc receptors, we hypothesized that the 5-HT_{1A}-receptor agonist, 8-OH-DPAT, would increase venous tone in rats subject to hypovolemic shock through sympathetic activation of α2-adrenergic receptors. Systemic administration of 8-OH-DPAT produced a sustained rise in blood pressure (+44 ± 3 mm Hg 35 min after injection, $P<0.01$ vs. saline) and mean circulatory filling pressure (+4.2 ± 0.7 mm Hg, $P<0.01$ vs. saline) in conscious rats subjected to hypovolemic shock. An equipressor infusion of epinephrine failed to influence mean circulatory filling pressure (MCFP). Ganglionic blockade, α1- or peripheral α2-adrenergic receptor blockade prevented the rise in MCFP observed with 8-OH-DPAT, but only α1-adrenergic receptor blockade diminished the pressor effect of the drug ($P<0.01$). 8-OH-DPAT raises blood pressure in rats in hypovolemic shock through both direct vascular activation- and sympathetic activation of α1-adrenergic receptors. The sympathoexcitatory effect of 8-OH-DPAT contributes to elevated venous tone through concurrent activation of both α1- and α2-adrenergic receptors. The data suggest that 5-HT_{1A} receptor agonists may provide an advantageous alternative to currently therapeutic interventions used to raise perfusion pressure in hypovolemic shock.

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Introduction

Progressive and severe blood loss elicits a complex series of autonomic responses that help to maintain or restore arterial blood pressure. During the initial phase of blood loss, arterial baroreflex-mediated increases in sympathetic drive help to maintain arterial pressure. If blood loss continues, these compensatory responses suddenly abate resulting in a syncopal-like episode characterized by low sympathetic activity and bradycardia (Schadt and Ludbrook, 1991). It is speculated that this latter phase may provide an adaptive means to increase cardiac filling and to help maintain cerebral perfusion (Oberg and Thoren, 1970;van Lieshout, et al., 2003). If hypotension persists, arterial baroreflex activity slowly recovers and progressive increases in sympathetic drive and tachycardia develop. The clinical features of this third phase of hemorrhage are commonly observed in patients who arrive in the emergency room after traumatic blood loss. Interventions at this stage must be rapid in order to prevent patients from progressing to a fourth, mostly irreversible stage of shock characterized by insensitivity to vasoconstrictors and high capillary permeability, both of which contribute to further maldistribution of blood volume and eventually death.

Rapid re-infusion of volume is a universally accepted treatment of hypovolemic shock. However, the type of resuscitation fluid used, as well as the amount and rate of re-infusion remain controversial. Also controversial is the choice of vasoconstrictor adjuvants used to help raise perfusion pressure. Epinephrine and other sympathomimetic agents are commonly given to support blood pressure during severe hypotensive shock when volume alone is insufficient to maintain pressure. However, catecholamine use is fraught with complications related to excessive vasoconstriction and exacerbation of

ischemia as well as generation of arrhythmias (Meier-Hellmann, et al., 1997). More recent evidence indicate that vasopressin and vasopressin analogues may be good alternatives to maintain arterial blood pressure in various types of shock (Kam, et al., 2004). While vasopressin is a highly potent arterial vasoconstrictor, it has virtually no vasoconstrictor effects on the venous vasculature (Warner, 1990). Theoretically, pressor agents that promote venous return and cardiac filling would provide a more favorable hemodynamic response than agents that act primarily by increasing arterial resistance. However, little is known about the venoconstrictor effects of pressor agents in hypovolemic shock.

We have shown that the 5-HT_{1A}-receptor agonists, (+)8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), produces a potent sympathoexcitatory response in conscious rats when administered during the syncopal phase of blood loss (Osei-Owusu and Scrogin, 2004b;Scrogin, 2003). Preliminary data also indicate that 8-OH-DPAT is an effective pressor agent when administered to rats in hypovolemic shock (Henze, et al., 2005). Venous tone is regulated largely by sympathetic drive (Pang, 2001). Therefore, we tested the hypothesis that 8-OH-DPAT increases arterial pressure during hypovolemic shock, in part, by stimulating sympathetic-mediated increases in venous tone through adrenergic receptor activation.

Methods

Animals

Male Sprague-Dawley rats weighing 310-360 gm (Harlan, Indianapolis, IN) were maintained in the institutional animal facility under standard conditions (22±2°C ambient temperature, 12:12 h light/dark cycle) with water and food provided *ad libitum*. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Surgery

Four days prior to experiments, rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p, Sigma) and instrumented with bilateral femoral arterial- and unilateral femoral venous polyethylene catheters for measurement of arterial pressure, arterial blood withdrawal and drug injections respectively. Silastic tubing (OD 0.037 in) was inserted into the thoracic vena cava via the femoral vein for measurement of the central venous pressure. A saline-filled inflatable balloon-tipped catheter (Vesta, Inc., Franklin, WI) was inserted into the right atrium via the jugular vein to allow brief cessation of circulation for measurement of mean circulatory filling pressure (MCFP), an indirect measure of venous tone. All catheters were tunneled under the skin to exit at the nape of a neck.

Experimental Protocols

Hemorrhage procedure

On the day of the experiment, animals were connected to the recording instrumentation while resting unrestrained in their home cage. Two measurements of

baseline MCFP were taken 20 and 10 minutes prior to initiation of hemorrhage according to methods developed by Yamamoto et al. (Yamamoto, et al., 1980). Hemorrhage was initiated using a modified Wigger's model. Blood was withdrawn at a rate of 3.2 ml/kg/min for 6 minutes, after which the rate was reduced to 0.53 ml/kg/min for an additional 4 minutes. Over the following 15 min, small amounts of blood (0.1-0.25 ml) were withdrawn or infused manually in order to maintain MAP at 50 mm Hg. All blood volume manipulations were terminated 25 min after initiation of hemorrhage, after which blood pressure was allowed to fluctuate. MCFP measurements were performed 20, 30, 40, 50, and 60 min after initiation of blood withdrawal.

Study 1

Animals were randomly assigned to one of 3 experimental groups all of which were given saline (150 µl, iv) 15 min after the initiation of blood withdrawal to control for volume of drug injections used in later protocols. Ten minutes later, immediately after the termination of blood withdrawal, animals were given 8-OH-DPAT (9.85 µg/kg/150 µl, iv, Research Biochemicals International) or saline. A third group received a variable infusion of epinephrine (2.5-1.0 µg/kg, Hospira, Inc), to match the blood pressure response of 8-OH-DPAT-treated rats. Arterial blood sampled 10 min after initiation of blood withdrawal (end of fixed-rate withdrawal) and 2 min after the last MCFP measurement were used for determination of hematocrit and total plasma protein concentration to assess the extent of hemodilution.

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Study 2

Rats were subject to the hemorrhage protocol described above, but were given the autonomic ganglionic blocker, hexamethonium chloride (30 mg/kg, iv, Sigma), 15 min after the initiation of hemorrhage, followed 10 min later by either saline or 8-OH-DPAT (9.85/kg/150 µl, iv).

Study 3

Rats were treated as in study 2 but were pretreated with the α_1-adrenergic receptor blocker, prazosin (25 µg/kg, iv, Sigma), rather than hexamethonium.

Study 4

Rats were treated as in study 2 except they were pre-treated with peripherally-acting α_2-adrenergic receptor blocker, L-659,066 (100 µg/kg, iv; (2R-*trans*)-*N*-(2-(1,3,4,7,12 b-hexahydro-2'-oxo-spiro(2 H-benzofuro(2,3-a)quinolizine- 2,4'-imidazolidin) –3'-yl)ethyl) methanesulphonamide monohydrochloride; Merck), rather than hexamethonium.

Data acquisition and analysis

During all experiments, arterial and central venous pressures (CVP) were recorded continuously on a Macintosh G4 PowerBook computer using PowerLab data acquisition software (Chart v.5.2.1, ADInstruments, Grand Junction, CO). Heart rate was calculated on line using peak-to-peak detection of the arterial pulse pressure wave.

Mean arterial pressure, HR and CVP were averaged within subject over 20 sec segments and averaged within groups at 5 min intervals.

Mean circulatory filling pressure (MCFP) was determined by initiating circulatory arrest by brief (~5sec) inflation of the balloon catheter. During balloon inflation, central venous pressure increased to a plateau level (VPP), while MAP decreased to a nadir, referred to as final arterial pressure (FAP). Mean circulatory filling pressure was calculated as VPP + 1/60(FAP – VPP). Total blood volume withdrawn during the course of hemorrhage was determined gravimetrically at the end of the hemorrhage period.

Two and 3-way ANOVAs with repeated measures were used to determine effects of autonomic manipulations and 8-OH-DPAT or epinephrine treatment over time (from 25 through 60 min after start of hemorrhage) on hemodynamic parameters where appropriate. Separate one and 2-way ANOVAs were used to assess effects of pressor agents on MCFP or the effects of hexamethonium-, prazosin- or L-659,066-treatment on MCFP responses to 8-OH-DPAT over time (from 20 through 60 min after start of hemorrhage). Significant main effects and interactions were followed up with Tukey/Kramer post-hoc tests. Total blood loss was pooled across pre-treatment groups. Total blood loss as well as change in hematocrit and plasma protein were analyzed by 2-way ANOVA followed by Tukey/Kramer post-hoc tests. Change in plasma protein and hematocrit of the two groups treated with L-659,066 were excluded from the ANOVA due to excessive variability.

Results

Statistical analyses showed no differences in hemodynamic responses to hemorrhage prior to drug treatment in the any of the groups. Thus, BP, HR and CVP data obtained prior to drug administration were pooled across groups prior to drug administration for clarity of presentation and to assess the hemodynamic profile during the initial blood withdrawal period.

Hemodynamic effect of 8-OH-DPAT in circulatory shock

The initial blood withdrawal (22.4 ml/kg) over the first 10 min of hemorrhage caused a precipitous drop in MAP (-70.4 ± 3.5 mmHg), HR (-138 ± 23 bpm) and CVP (-1.5 ± 0.5 mmHg). An additional 12.2 ± 1.0 ml/kg of blood was withdrawn over the following 15 min in order to maintain MAP at 50 mmHg. Heart rate reached a nadir 10 min after initiation of blood withdrawal, but then began to rise steadily and stabilized near baseline by the end of blood withdrawal. Changes in CVP paralleled changes in MAP (Figure 1).

Following termination of hemorrhage, 8-OH-DPAT administration caused a rapid rise in MAP that persisted throughout the 35 min post-hemorrhage recording period. Heart rate and CVP were not affected by 8-OH-DPAT. Continuous infusion of epinephrine, titrated to match the pressor effect of 8-OH-DPAT caused a distinct tachycardia during the early part of the infusion (Figure 1).

Balloon inflation prior to hemorrhage caused a large rise in CVP. In subsequent tests after blood loss the rise in CVP was markedly attenuated and remained low throughout the post hemorrhage period in saline-treated rats. The rise in CVP during

balloon inflation was exaggerated in hemorrhaged rats given 8-OH-DPAT (data not shown). This resulted in a significant elevation of MCFP that lasted throughout the recovery period. Epinephrine infusion had no effect on MCFP (Figure 2).

Effect of Autonomic Blockade on hemodynamic responses to 8-OH-DPAT

Ganglionic blockade caused an immediate drop in pressure below the target MAP of 50 mmHg (data not shown). This was quickly rectified by re-infusion of a small amount of shed blood. As a result, the total blood withdrawal needed to sustain hypotension was reduced in rats given hexamethonium (Table 1). Ganglionic blockade had a slight, but non-significant tachycardic effect and did not influence CVP (data not shown).

Ganglionic blockade attenuated recovery of blood pressure following termination of hemorrhage. However, the immediate pressor response to 8-OH-DPAT was similar in intact and ganglionic-blocked rats when compared to their respective control groups (Figure 3, compare light and dark gray shaded areas). With time, the pressor response diminished in ganglionic-blocked animals (darker gray), but grew larger in intact animals (light gray). An overall ANOVA revealed a significant interaction between Ganglionic Blockade, 8-OH-DPAT Treatment and Time ($P<0.01$). Subsequent 2-way ANOVAs performed at each time point showed significant interactions between Ganglionic Blockade and 8-OH-DPAT 50 and 55 min after the start of hemorrhage due to the waning pressor effect of 8-OH-DPAT after ganglionic blockade and the persistent pressor effect in intact animals. Heart rate and CVP were not significantly affected by 8-OH-DPAT in either intact- or ganglionic-blocked rats (data not shown).

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MCFP is directly affected by both blood volume and venous tone (Guyton, et al., 1954). Since the total volume of blood withdrawn differed among rats given various pre-treatments (i.e, hexamethonium, prazosin, L-659,066 or saline), MCFP was only compared between groups subjected to similar degrees of blood withdrawal, e.g., in study 2, only 8-OH-DPAT- and saline-treated rats subjected to ganglionic blockade were compared to one another, while animals with intact autonomic responses were compared in a separate analysis. In contrast to intact rats, 8-OH-DPAT did not increase MCFP after ganglionic blockade (Figure 4).

Effect of Prazosin on 8-OH-DPAT-mediated hemodynamics

Blockade of peripheral α_1-adrenergic receptors exacerbated the hemorrhage-induced hypotension resulting in less blood withdrawal over the course of hemorrhage (Table 1). Prazosin also attenuated recovery of blood pressure following termination of hemorrhage and completely blocked the pressor effect of 8-OH-DPAT (Figure 5). Prazosin had no effect on either HR or CVP (data not shown), but blocked the ability of 8-OH-DPAT to increase MCFP (Figure 6).

Effect of L-659,066 on 8-OH-DPAT-mediated hemodynamics

Blockade of peripheral α_2-adrenergic receptors did not alter blood pressure prior to saline or 8-O-DPAT administration. Consequently, total blood loss did not differ between rats pre-treated with the α_2-adrenergic receptor antagonist and those pre-treated with saline (Table 1). However, α_2-adrenergic receptor blockade did attenuate the recovery of blood pressure following termination of blood withdrawal and tended to

accelerate decompensation in a subset of animals leading to the increased blood pressure variability observed at the end of the recording period. The magnitude of the initial pressor response to 8-OH-DPAT was not affected by α_2-receptor blockade. Though the blood pressure profile observed after L-659,066 clearly resembled that observed after hexamethonium, there was no significant interaction between L-659,066 and 8-OH-DPAT treatment either as a whole or over time. L-659,066 raised HR and CVP immediately after injection, but did not influence the HR or CVP response to 8-OH-DPAT (Figure 7).

L-659,066 pre-treatment blocked the effect of 8-OH-DPAT on MCFP (Figure 8). L-659,066 itself appeared to lower MCFP compared to control animals despite a similar volume of blood withdrawal during hemorrhage (compare Figures 2 and 8). However, hematocrit and plasma protein changes were highly variable in animals treated with L-659,066. Therefore, MCFP of saline- and L-659,066-treated rats was not directly compared.

Discussion

In the current study, 8-OH-DPAT elicited a significant pressor response when administered during hypovolemic shock. The pressor effect was mediated by a combination of direct- and sympathetic-dependent activation of α_1-adrenergic receptors. 8-OH-DPAT produced a similar initial pressor effect in the absence of ganglionic blockade suggesting that the direct vascular effect of 8-OH-DPAT predominated immediately after drug treatment. The pressor effect waned after 20-25 min in animals

subjected to ganglionic blockade, but persisted in intact animals, indicating that the sympathetic component of the pressor response elicited a lasting hemodynamic effect.

Sympathetic-dependent activation of α_1-adrenergic receptors mediated a significant amount of the compensatory vasoconstriction that developed following termination of blood withdrawal in control animals. This was evidenced by the lower blood pressure observed in prazosin-treated rats following termination of blood withdrawal despite their having had significantly less blood withdrawn than control animals during hemorrhage. Vascular α_2-adrenergic receptors also contributed to compensation during recovery. However, the effect was not immediate as the α_2-adrenergic antagonist, L-695,099, did not reduce the volume of blood withdrawal necessary to maintain pressure during active hemorrhage. After hemorrhage termination, blood pressure of α_2 antagonist-treated rats began to fall towards the end of the recording period. In fact, several animals pre-treated with the α_2-antagonist alone tended to develop what appeared to be the beginning of irreversible decompensation prior to the end of the recording period. 8-OH-DPAT protected against this effect suggesting that sympathetic activation of α_1-receptors may compensate for lack of α_2 -receptor activation to maintain blood pressure.

8-OH-DPAT markedly elevated MCFP through an autonomic-dependent mechanism. MCFP is determined by total blood volume and overall vascular compliance. Thus, MCFP is primarily dependent on volume and venous tone since venous compliance is so much larger than arterial compliance (Guyton, et al., 1954). Thus, 8-OH-DPAT mediated its effects on MCFP in hemorrhaged animals either by increasing vascular blood volume, venous tone or both. Neither the volume of blood

withdrawn, nor the hemorrhage-induced change in hematocrit differed between 8-OH-DPAT- and saline-treated control groups suggesting that differences in capillary refilling contributed little to the difference in MCFP. However, it cannot be ruled out that the assessment of hemodilution differences was confounded by a sympathetic-mediated increase in erythrocyte release by splenic contraction in 8-OH-DPAT-treated animals (Kuwahira, et al., 1999). However, plasma protein declined to the same degree in animals treated with 8-OH-DPAT and saline, favoring the view that increases in MCFP were primarily mediated by increased venous tone.

The rise in MCFP was prevented by either α_1- or α_2-receptor blockade suggesting that both receptor subtypes must be available in order for 8-OH-DPAT to increase venous tone during hypovolemic shock. In accord, previous studies have shown that treatment with either prazosin or rauwolscine produces a dose-dependent decrease in MCFP in euvolemic conscious rats, but only during reflex sympathetic activation (D'Oyley and Pang, 1990). Prazosin was also found to reduce MCFP in an anesthetized, open chest dog preparation, but only during infusion of norepinephrine (Ito and Hirakawa, 1984). Selective α_1-adrenergic agonists produce little increase in MCFP when infused into intact rats, while norepinephrine produces a potent, dose-dependent increase in MCFP (Pang and Tabrizchi, 1986). Studies in isolated mesenteric veins confirm that activation of α_1-adrenergic receptors may be necessary to observe a venoconstrictor effect of α_2-adrenergic receptors in some vascular beds. Specifically, clonidine and other α_2-adrenergic agonists alone do not produce mesenteric venoconstriction, but yohimbine, idazoxan or rauwolscine inhibit venoconstriction caused by norepinephrine (Greg Fink, Ph.D., unpublished data, 2006). Taken together the data suggest that α_1- and α_2-

adrenergic receptor populations interact with one another to mediate sympathetic-dependent increases in venous tone.

Surprisingly, epinephrine did not affect MCFP despite its high affinity and agonist activity at both α_1- and α_2-adrenergic receptor subtypes. To our knowledge, the effect of epinephrine on MCFP has not previously been studied in mammals. Selective β-receptor agonists tend to increase MCFP in intact rats, but produce a consistent decrease in MCFP when administered after blockade of sympathetic reflexes (Abdelrahman and Pang, 1990). Thus, the β-adrenergic properties of epinephrine may have antagonized its α-adrenergic mediated venoconstrictor effect. Alternatively, the venoconstrictor effect of epinephrine may have been masked by a concomitant loss of circulating blood volume due to increased capillary filtration. Though not significant, the fall in hematocrit tended to be larger with epinephrine infusion arguing against a greater loss of intravascular volume in this group.

Presumably, a peripherally acting agent with both α_1 and α_2 agonists activity, but little β_2 activity such as norepinephrine would significantly increase venous tone during hemorrhage. As described above norepinephrine has a potent effect on MCFP in intact animals. To our knowledge, no one has yet determined the effect of norepinephrine on MCFP in hemorrhage. Such studies are problematic because of norepinephrine's propensity to exacerbate ischemia during hypovolemia. There were no outward signs of exacerbated ischemia in animals treated with 8-OH-DPAT. In accord, preliminary evidence suggests that the 8-OH-DPAT does not exacerbate ischemic end-organ injury as assessed by hemorrage-induced neutrophil activation in lung, kidney or gut (Osei-Owusu and Scrogin, 2004a).

An increase in venous tone should result in an elevation in venous return and a rise in blood pressure if right atrial pressure is maintained. Surprisingly, neither ganglionic blockade nor α_2-receptor blockade had any apparent effect on the initial pressor response to 8-OH-DPAT despite their ability to block the 8-OH-DPAT-mediated rise in MCFP. However, it could be argued that the direct arterial vasoconstrictor effect of 8-OH-DPAT was exaggerated after ganglionic blockade due to the greater availability of adrenergic receptors. Pressor responses to norepinephrine are exaggerated in euvolemic animals after ganglionic blockade (Del Basso, et al., 1983;Rowe, et al., 1979). The pressor response to 8-OH-DPAT-mediated sympathetic activation was also likely exaggerated during α_2-receptor blockade due to lack of α_2-adrenergic autoreceptor inhibition of catecholamine release. This view is supported by the greater HR rise observed during blood withdrawal in animals treated with L-659,066. Thus, we suspect that the rise in MCFP elicited by 8-OH-DPAT does indeed contribute to increased venous return via its sympathoexcitatory action.

The similarity in the blood pressure profiles of animals treated with 8-OH-DPAT following ganglionic blockade and α_2-adrenergic antagonist administration suggests that the late pressor effect of 8-OH-DPAT was mediated largely by sympathetic activation of α_2-adrenergic receptors. Animals subjected to ganglionic blockade, however, did not show the late decompensatory response seen after selective α_2-adrenergic blockade. However, it should be recognized that rats treated with the ganglionic blocker were subjected to significantly less blood withdrawal than those treated with the α_2-recpetor antagonist.

It is possible that MCFP was increased by mobilization of blood stores following 8-OH-DPAT-medated increases in sympathetic drive. Sympathetic activation produces a prolonged, slow contraction of the spleen in rats (Kuwahira, et al., 1999). The rat spleen is innervated by sympathetic nerves and expresses a high density of α_2-adrenergic receptors (Handy, et al., 1993). Moreover, α_2-adrenergic receptor antagonists interfere with hypoxia induced increases in hematocrit proposed to result from splenic contraction in the rat (Kuwahira, et al., 1999). It has been proposed that the rat spleen, like that of the human, contributes to intravascular blood volume regulation primarily through diversion of cell free filtrate to the lymphatic system, most likely through reflex sympathoinhibition stimulated by cardiopulmonary stretch (Kaufman and Deng, 1993). 8-OH-DPAT could exaggerate sympathetic activation during hypovolemia thus attenuating splenic filtration. The lack of difference in hematocrit fall after 8-OH-DPAT would argue against this view. Nevertheless, the delayed decompensatory effect of the α_2-adrenergic receptor antagonist observed in the current study may reflect blockade of a relatively slow contribution of the spleen or other splanchnic organs to venous return.

The slow rise in heart rate observed over the duration of active hemorrhage suggests that a parallel increase in sympathetic drive also likely occurs. Our preliminary studies indicate that renal sympathetic activity rises in parallel with heart rate in this model of hypovolemic shock (data not shown). It is tempting to speculate that 8-OH-DPAT accelerates the sympathetic-mediated fluid redistribution that normally occurs during compensation. The rapid rise in sympathetic activity appears to provide a superior hemodynamic response compared to that elicited by epinephrine infusion. Like 8-OH-DPAT, hypertonic saline resuscitation has also been found to increase sympathetic

activation and promote favorable hemodynamic responses compared to vasoconstrictor infusion (Seki, et al., 1997).

The data herein indicate that 5-HT_{1A} agonist may provide an advantageous alternative to direct vasoconstrictors in the treatment of hypovolemic shock, in part, because it mobilizes existing blood stores. An additional advantage of 8-OH-DPAT is its relatively long half-life (~20 min), making it amenable to single dosing during emergent resuscitation outside of the hospital. 5-HT_{1A}-receptor agonists also produce mild hypothermic effects and stimulate the hypothalamo-pituitary adrenal axis, and thus glucocorticoid release, suggesting they may combate ischemia reperfusion injury (Cleare, et al., 1998;Shiah, et al., 1998). 5-HT_{1A}-receptor agonists are also neuroprotective during cerebral ischemia, presumably due to their ability to inhibit glutamatergic neurotoxicity (Bielenberg and Burkhardt, 1990;Semkova, et al., 1998). These characteristics, together with the data provided here suggest that 5-HT_{1A}-receptor agonists may provide a beneficial alternative to currently used vasoconstrictors to raise pressure during hypovolemic shock.

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Footnotes

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Legends for Figures

Figure 1. Mean arterial pressure (MAP), heart rate (HR) and central venous pressure (CVP) during hemorrhage (duration indicated by shaded box) and subsequent injection of 8-OH-DPAT, saline or epinephrine infusion. Data are groups means ± S.E. Data prior to drug injection are pooled. Group n's indicated in parentheses. $^{**}P<0.01$ 8-O-DPAT vs. saline, $^{\dagger\dagger}P<0.01$ Epinephrine vs. saline, $^{\S}P<0.05$ Epinephrine vs. 8-OH-DPAT. All groups were given saline pre-treatment as a control for other pretreatments used in subsequent studies.

Figure 2. Mean circulatory filling pressure (MCFP) 10 min prior to hemorrhage and 20, 30, 40 ,50 and 60 min after start of blood withdrawal (shaded box) in animals treated with saline, 8-OH-DPAT or epinephrine. Data are group means ± S.E. Group n's are in parentheses. $^{**}P<0.01$ vs. saline, $^{+}P<0.05$ vs. epinephrine.

Figure 3. Mean arterial pressure (MAP) during hemorrhage (shaded box) and subsequent administration of hexamethonium chloride (Hex) or saline (15 min), followed by either 8-OH-DPAT or saline (25 min). Data are pooled prior to first injection, then divided into Hex- (closed triangles) and saline-treated (closed circles) groups following the first injection and further divided into the 4 final groups following the second injection. Shading delineates pressor response to 8-OH-DPAT with respect to its appropriate

control group in intact (light gray) and ganglionic blocked (dark gray) animals. Darkest shading indicates overlap between pressor responses. Intact 8-OH-DPAT- and saline-treated group data taken from experiment 1 are included in analysis. Data are groups means ± S.E. Groups n's are in parentheses. $^{**}P<0.01$ Saline + 8-OH-DPAT vs. Saline + Saline, $^{\S\S}P<0.01$ Saline + 8-OH-DPAT vs. Hex + 8-OH-DPAT, $^{\#,\#\#}P<0.05$, 0.01 Hex + 8-OH-DPAT vs. Hex + Saline, $^{++}P<0.01$ Hex + Saline vs. Saline + Saline.

Figure 4. Mean circulatory filling pressure (MCFP) prior to hemorrhage and 20, 30 ,40, 50 and 60 min after start of blood withdrawal in animals given saline or 8-OH-DPAT following ganglionic blockade with hexamethonium chloride. Data are group means ± S.E. Group n's indicated in parentheses of legend.

Figure 5. Mean arterial pressure (MAP) during hemorrhage and subsequent administration of prazosin (Prz) or saline (15 min), followed by either 8-OH-DPAT or saline (25 min). Data are pooled prior to first injection, then divided into prazosin- (closed triangles) and saline-treated (closed circles) groups following the first injection and further divided into the 4 final groups following the second injection. Shading represents pressor response to 8-OH-DPAT with respect to its appropriate control group in intact (light gray) and prazosin pre-treated (dark gray) animals. The intact 8-OH-DPAT- and saline-treated group data are included from experiment 1. Data are group means ± S.E. Group n's indicated in parentheses. $^{**}P<0.01$ Saline + 8-OH-DPAT vs. Saline + Saline, $^{\S\S}P<0.05$ Saline + 8-OH-DPAT vs. Prz + 8-OH-DPAT, $^{+,++}P<0.05$, 0.01 Prz + Saline vs. Saline + Saline.

Figure 6. Mean circulatory filling pressure (MCFP) prior to hemorrhage and 20, 30 ,40, 50 and 60 min after start of blood withdrawal in animals given saline or 8-OH-DPAT following prazosin. Data are group means ± S.E. Group n's provided in parentheses in legend.

Figure 7. Mean arterial pressure (MAP) during hemorrhage and subsequent administration of the α2-adrenergic receptor antagonist, L-659,066, or saline (15 min), followed by either 8-OH-DPAT or saline (25 min). Data are pooled prior to first injection, then divided into L-659,066 (closed triangles) and saline-treated (closed circles) groups following the first injection, then divided further into the 4 final groups following the second injection. Shading represents pressor response to 8-OH-DPAT with respect to its appropriate control group in intact (light gray) and L-659,066-treated (dark gray) animals. Darkest gray indicates overlap between pressor responses. The intact 8-OH-DPAT- and saline-treated group data are included from experiment 1. Data are group means ± S.E. ‡,‡‡ P<0.05, 0.01, L-659,066 vs. saline (prior to second injection). **P<0.01 Saline + 8-OH-DPAT vs. Saline + Saline, $^{+,++}P$<0.05, 0.01 L-659,066 + Saline vs. Saline + Saline. There was no interaction between 8-OH-DPAT and L-659,066 treatment so differences are not reported

Figure 8. Mean circulatory filling pressure (MCFP) prior to hemorrhage and 20, 30 ,40, 50 and 60 min after start of blood withdrawal in animals given saline or 8-OH-DPAT

following L-659,066. Data are group means ± S.E. Group n's provided in parentheses in legend.

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Table 1. Blood Volume Parameters

	Total blood loss (ml/kg)	Δ Hematocrit (%)	Δ Plasma protein (g/100 ml)
Saline + Saline (10)		-3.9±0.9	-0.57±0.10
Saline + 8-OH-DPAT (10)	35.0 ± 0.10	-3.6±0.9	-0.47±0.10
Epinephrine (8)		-6.3±1.2	-0.61±0.06
Hex + Saline (8)	29.6 ± 0.10**††	-5.5±0.5	-0.63±0.07
Hex + 8-OH-DPAT (8)		-5.9±0.7	-0.60±0.06
Prazosin + Saline (8)	25.9 ± 0.10**††	-6.3±1.0	-0.56±0.10
Prazosin + 8-OH-DPAT (9)		-4.4±0.7	-0.43±0.13
L-659,066 + Saline (7)	34.3 ± 0.04	-1.0±1.8	+0.17±0.37
L-659,066 + 8-OH-DPAT (7)		-1.0±1.7	-0.04±0.31

Values are mean ± S.E. Blood loss data are pooled across pre-treatment groups. Hematocrit and plasma protein determined 10 min after initiation of blood withdrawal and end of recovery, 60 min after start of hemorrhage.

**P<0.01 vs. saline pre-treatment, ††P<0.01 vs. L-659,066 pre-treatment.

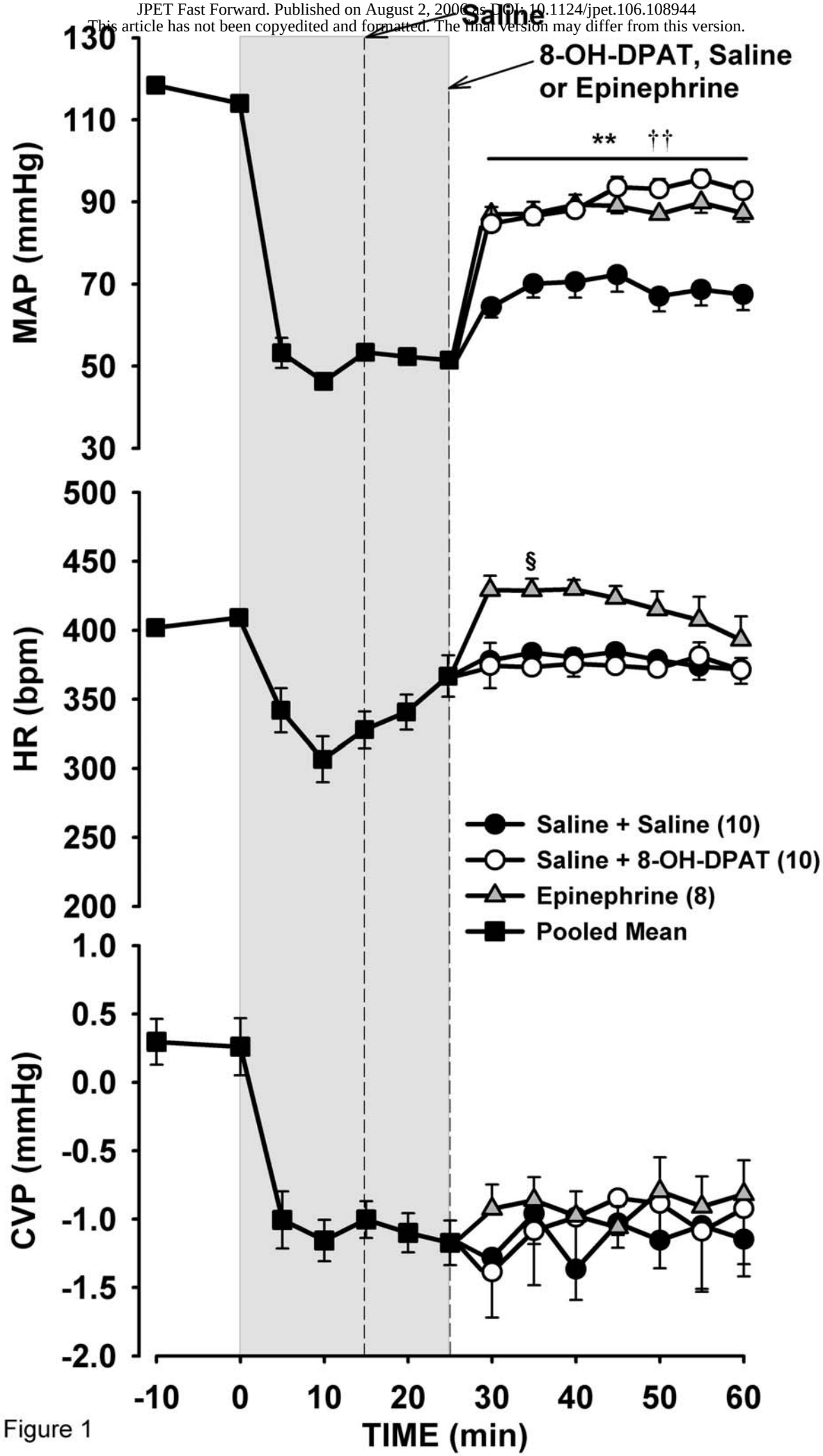

Figure 1

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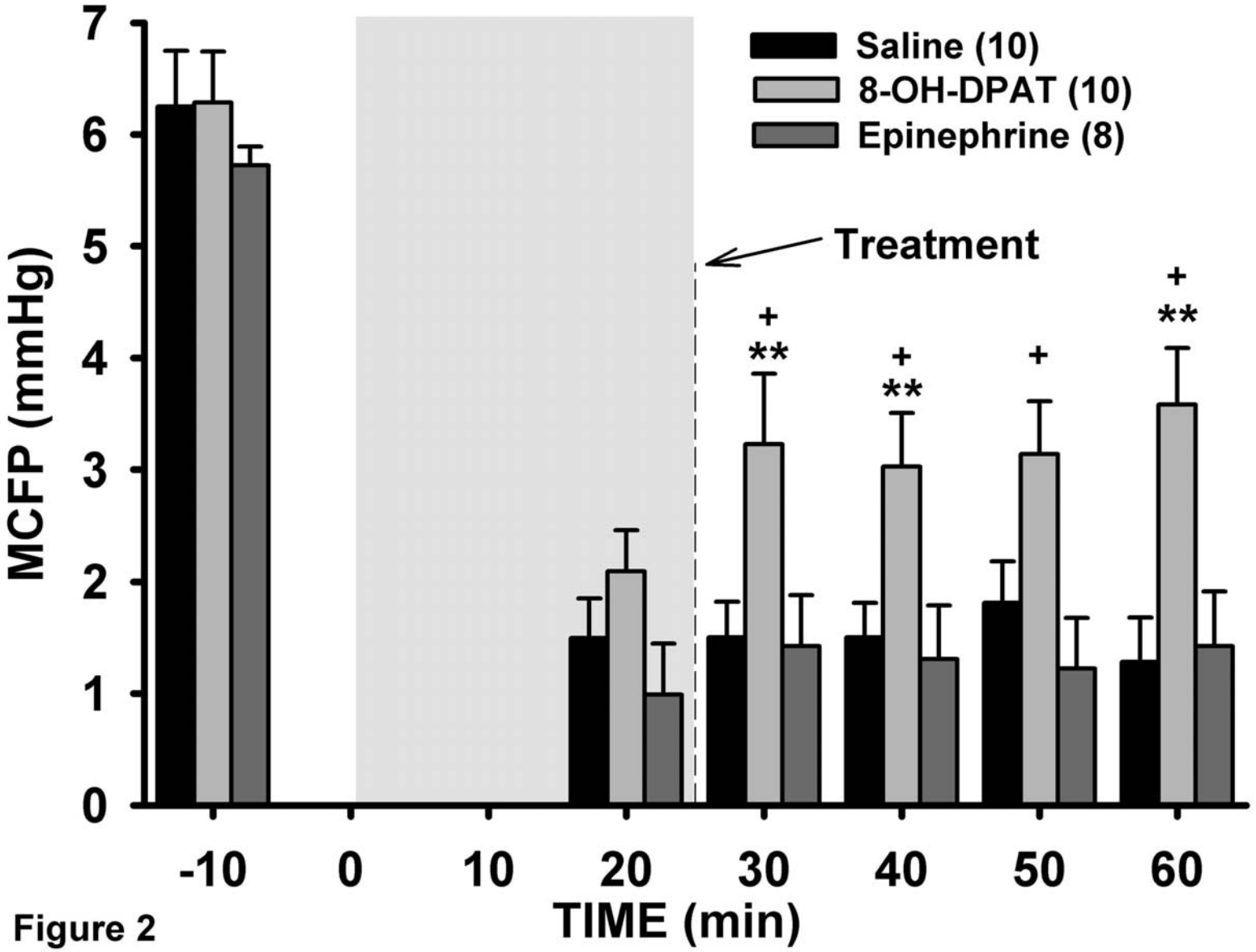

Figure 2

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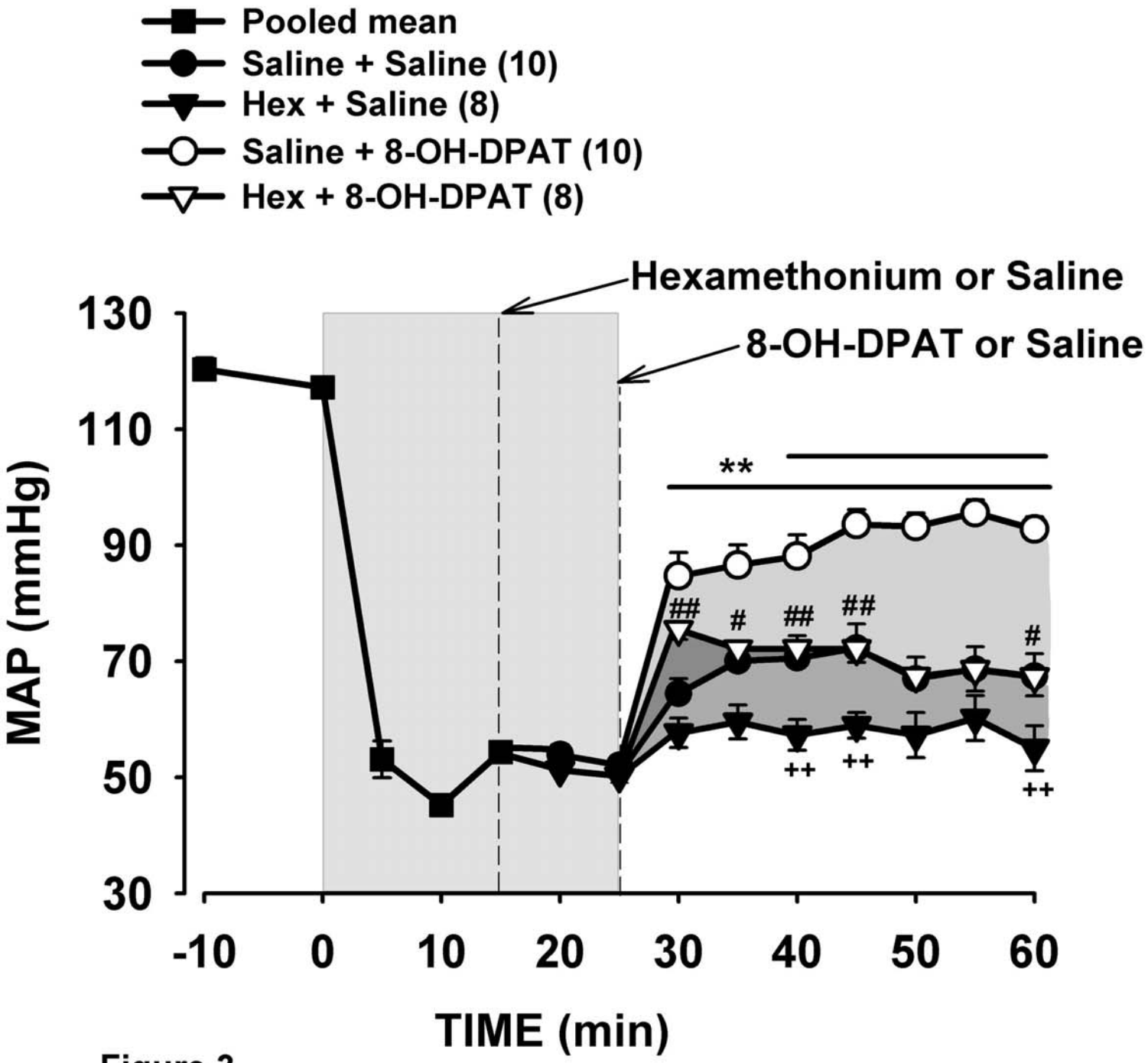

Figure 3

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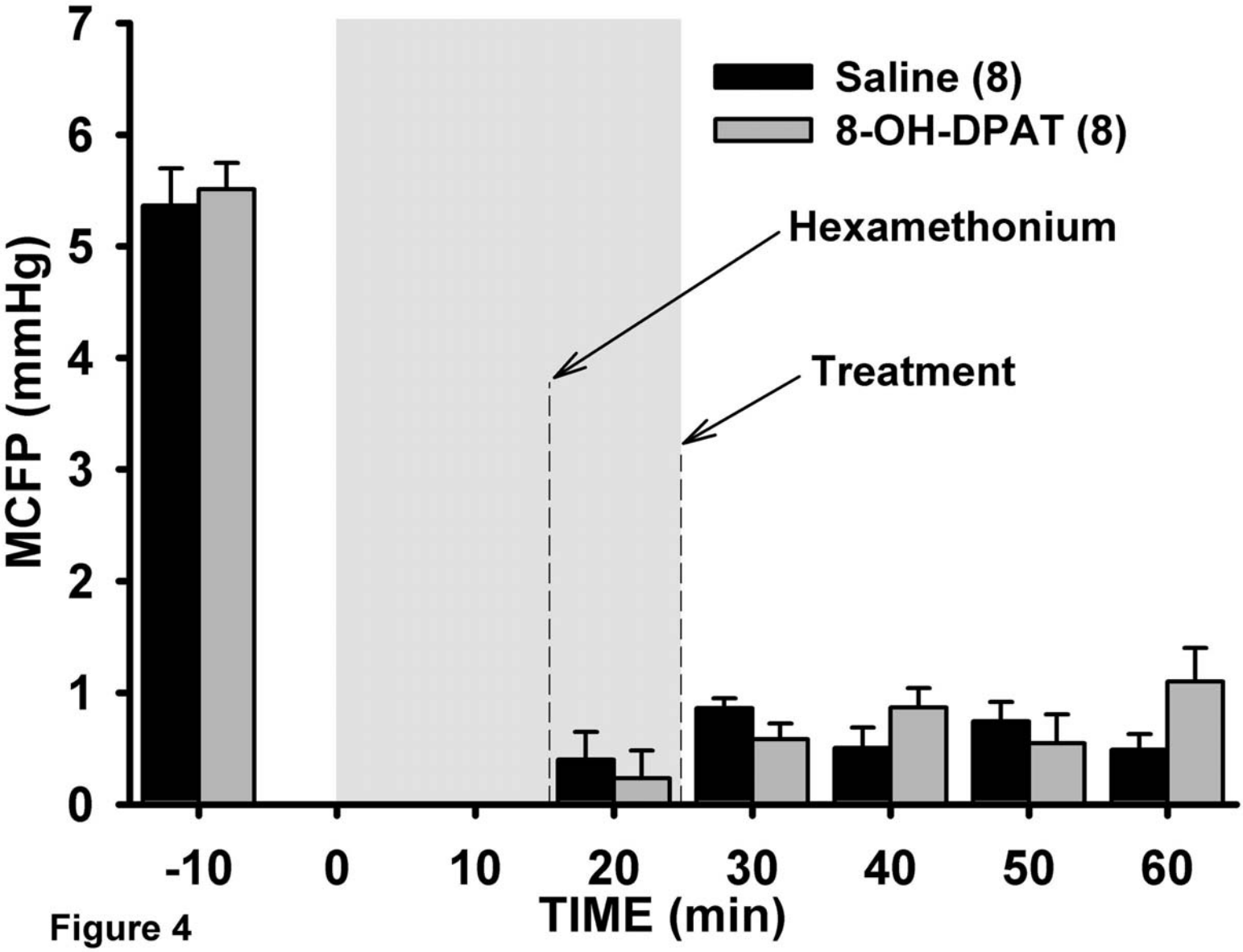

Figure 4

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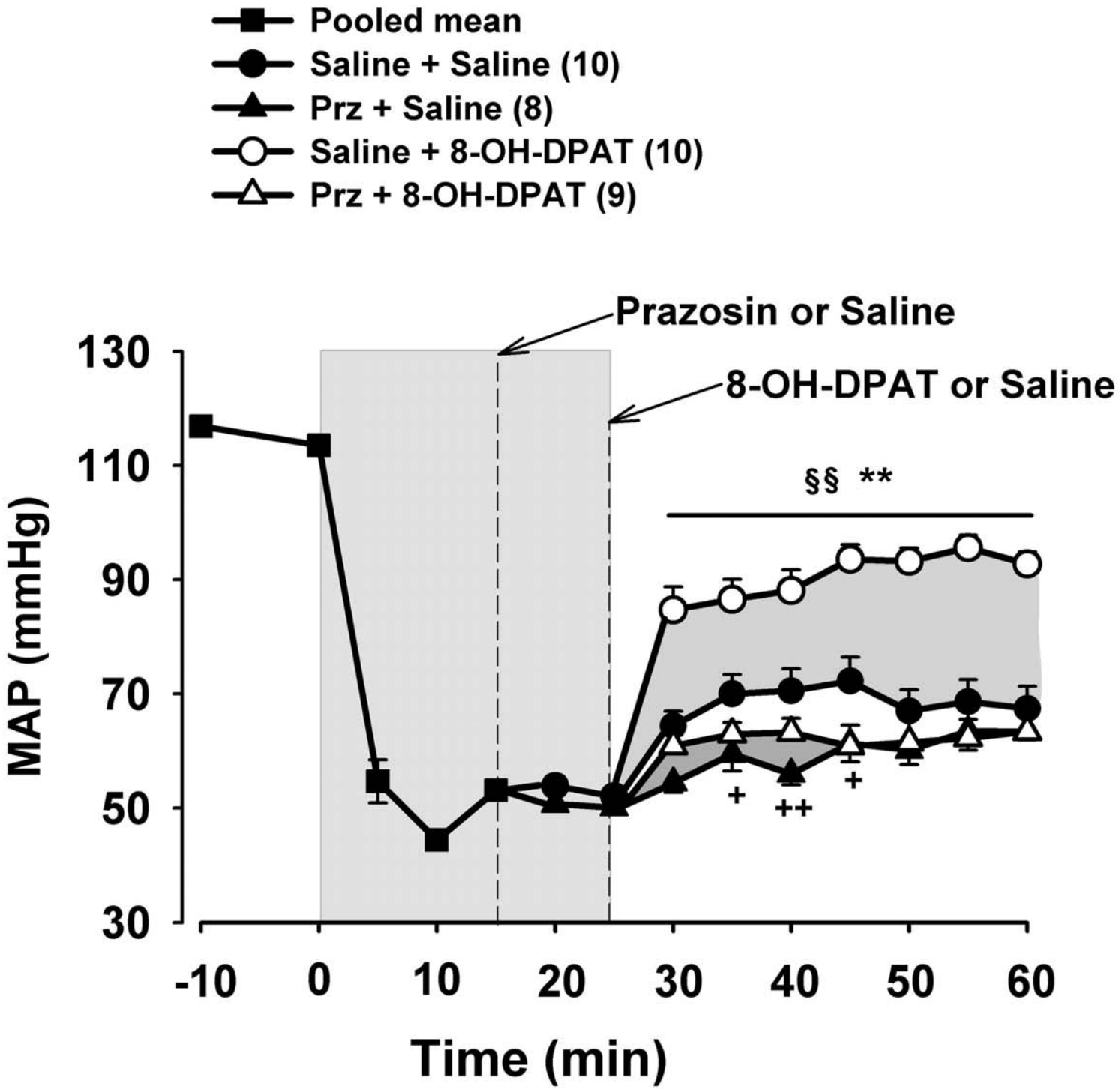

Figure 5

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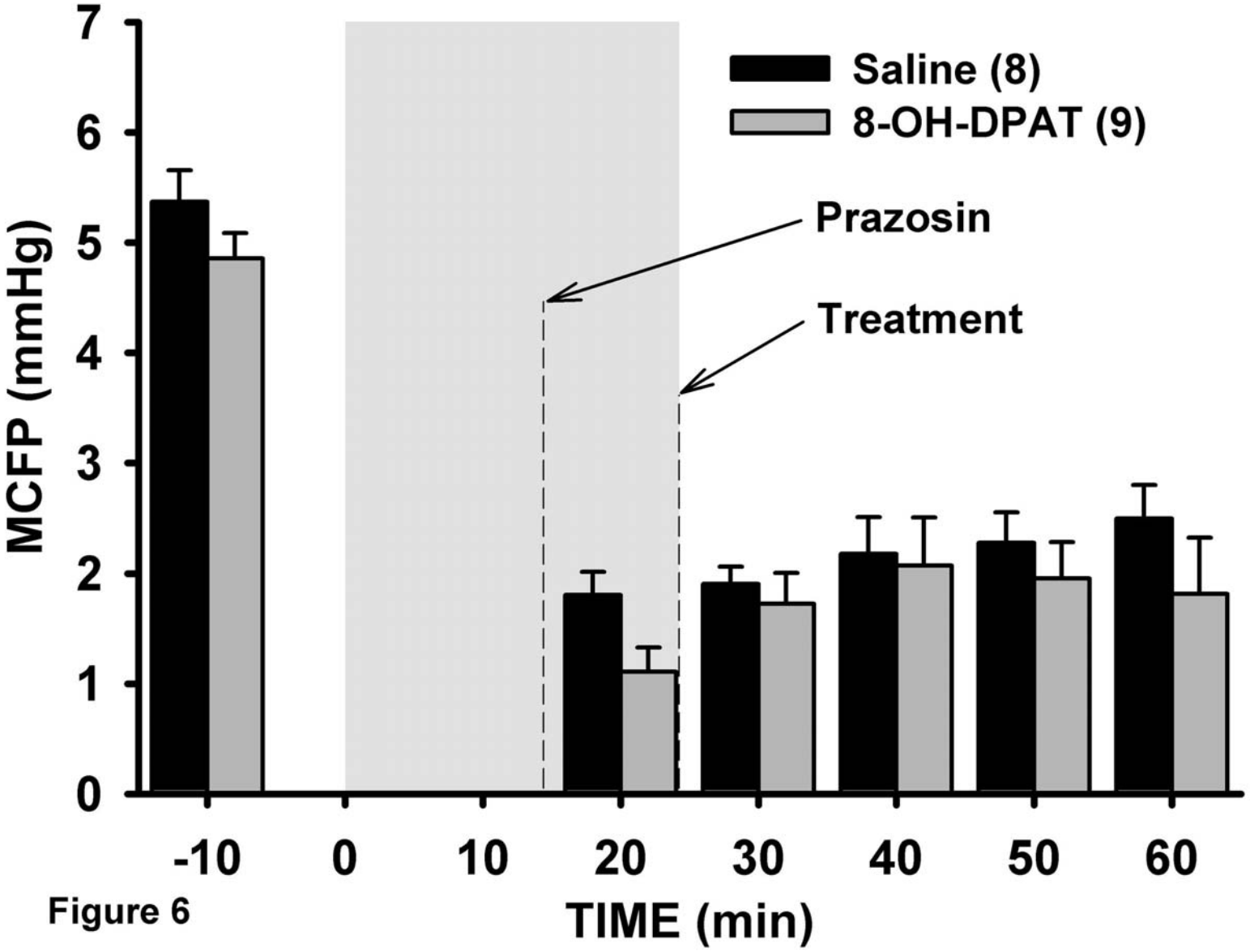

Figure 6

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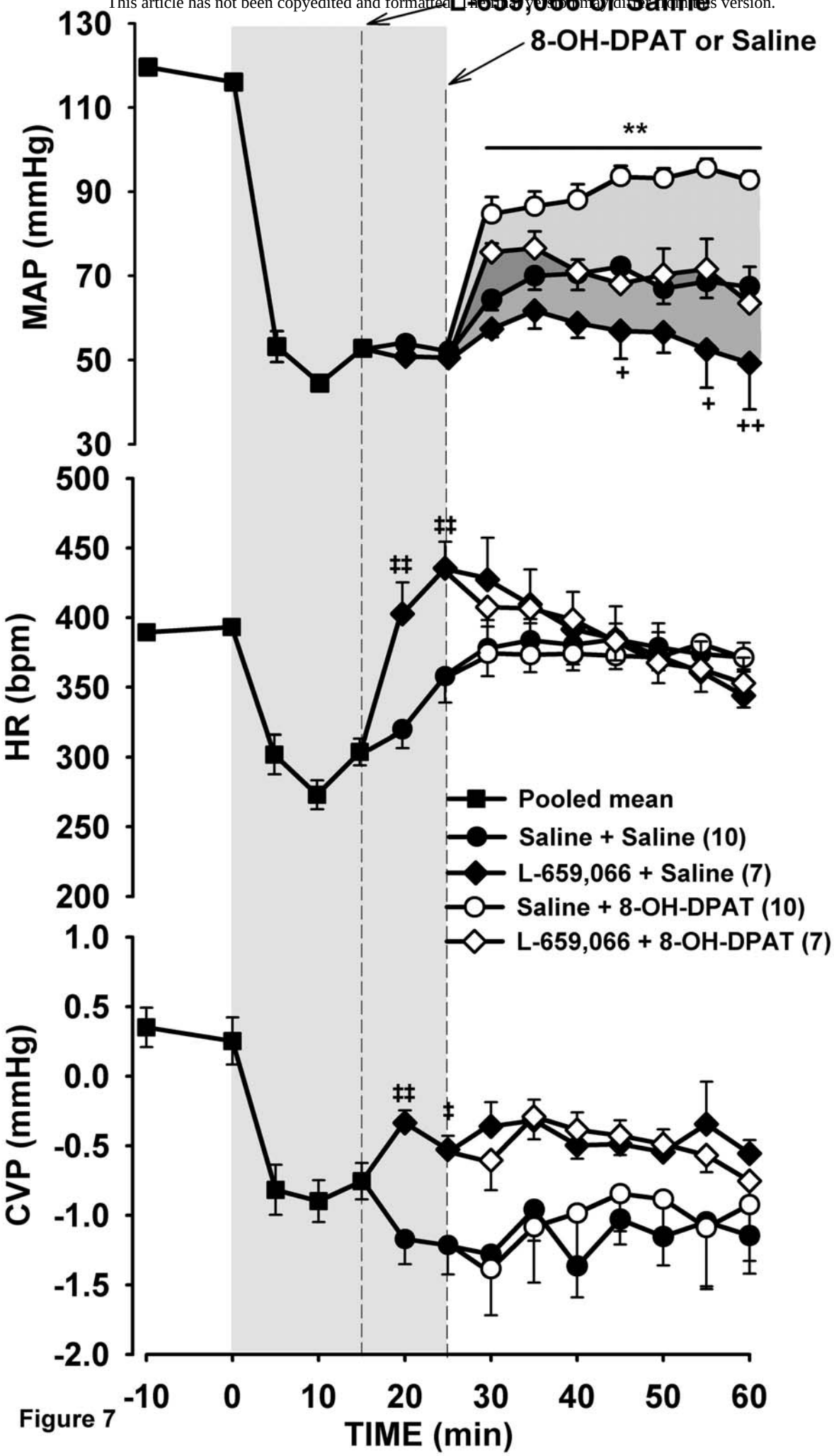

Figure 7

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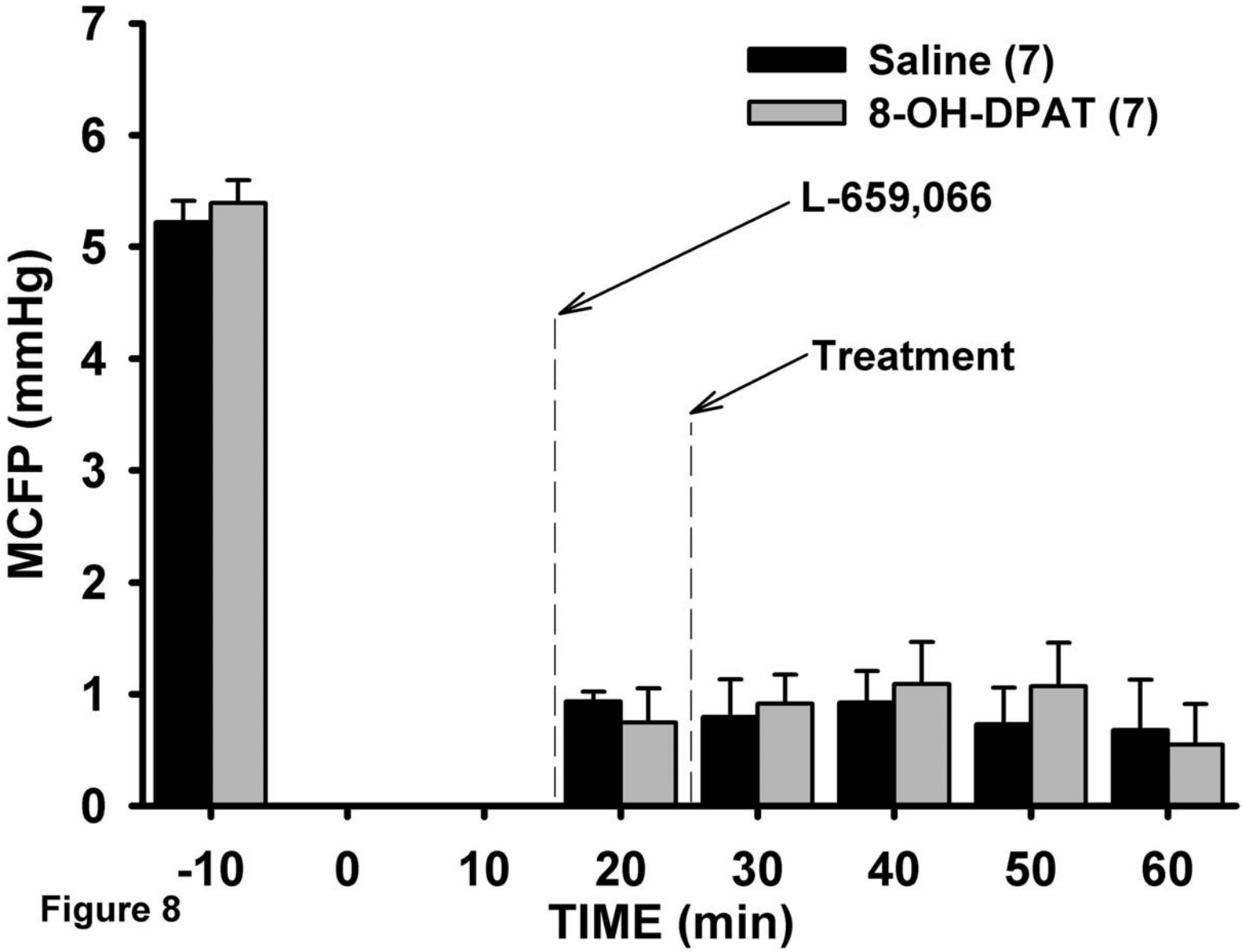

Figure 8