

CYTIDINE 5'-DIPHOSPHOCHOLINE RESTORES BLOOD FLOW OF SUPERIOR MESENTERIC AND RENAL ARTERIES AND PROLONGS SURVIVAL TIME IN HAEMORRHAGED ANAESTHETIZED RATS

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SUMMARY

1. The aim of the present study was to investigate the effect of the intracerebroventricular (i.c.v.) or intravenous (i.v.) administration of cytidine 5'-diphosphocholine (CDP-choline) on superior mesenteric artery (SMA) and renal artery (RA) blood flow, along with the cardiovascular parameters and survival time of anaesthetized rats under conditions of haemorrhagic shock.

2. Rats were anaesthetized with urethane (1.25 g/kg, i.p.) and acute haemorrhage was mimicked by the withdrawal of a total volume of 2–2.1 mL blood/100 g bodyweight over a period of 20 min. The CDP-choline was injected i.c.v. (1.0, 1.5 and 2.0 μ mol) or i.v. (250 mg/kg) after the end of haemorrhage. Blood pressure, heart rate, SMA and RA flow values and the survival time of rats were recorded. Changes in blood flow were estimated by laser-Doppler flowmetry.

3. The haemorrhage procedure decreased the blood pressures of rats by 60% and limited their survival time to 22 ± 2 min. Both SMA and RA flow decreased to approximately 25% of initial values at the end of the haemorrhage procedure.

4. The i.c.v. administration of CDP-choline (1.0, 1.5 and 2.0 μ mol) increased blood pressure and partially reversed the hypotension in a dose- and time-dependent manner. At 1.5 and 2.0 μ mol, i.c.v., CDP-choline completely restored the decreased flow of the RA and transiently reversed hypoperfusion of the SMA. It also produced an almost fourfold increase in the survival time of rats.

5. The i.v. administration of CDP-choline (250 mg/kg) also completely, but transiently, restored SMA and RA flow, whereas it increased blood pressure by only 40% compared with control values. The survival time of rats in the i.v. CDP-choline group was doubled that of control.

6. These results indicate that both centrally and peripherally injected CDP-choline can restore SMA and RA flow, together with a partial reversal of hypotension and an increase in the survival time of rats.

Key words: blood flow, cytidine 5'-diphosphocholine, hypotension, renal artery, shock, superior mesenteric artery, survival.

INTRODUCTION

Cytidine 5'-diphosphocholine (CDP-choline; citicoline) is an endogenously synthesised mononucleotide. It exerts variety of beneficial physiological and pharmacological effects on body functions by mediating membrane phospholipid production,^{1,2} by altering brain membrane metabolism under ischaemic conditions^{3,4} and by increasing central cholinergic^{5,6} and dopaminergic transmission.⁷ It is also a drug used for the treatment of brain injury and stroke in several European countries and Japan, with almost no toxicity.^{4,8} We demonstrated recently that intracerebroventricular (i.c.v.)⁵ or intravenous (i.v.)⁶ injection of CDP-choline very effectively increases blood pressure in normal rats and reverses hypotension under conditions of haemorrhagic shock. Shock is characterized by hypotension and hypovolaemia leading to the deterioration of the perfusion of several tissue and organ systems.^{9,10} It is known that haemorrhagic shock can cause anuria and renal failure by profoundly decreasing renal blood flow¹¹ and that it generates selective mesenteric ischaemia by producing a disproportionate mesenteric vasospasm, which is one of the most important reasons for the decreased survival under these conditions.^{12–14} Furthermore, it has been reported that the mesenteric vasoconstrictor response to haemorrhage is mediated primarily by the activation of the renin–angiotensin axis.¹² Cytidine 5'-diphosphocholine does not affect plasma renin activity, whereas it does increase plasma vasopressin and adrenaline levels during its reversal of hypotension.^{5,6} Hence, we postulated that CDP-choline would be able to improve the decreased blood flow of the superior mesenteric and renal arteries (SMA and RA, respectively), together with a restoration of blood pressure without changing renin activity, and that this effect would be advantageous in determining the outcome of hypovolemic shock. The present study was designed to determine whether CDP-choline can affect the flow of those important arteries, as well as survival time after haemorrhagic shock, by increasing blood pressure in anaesthetized rats.

METHODS

Experimental animals

Adult male Wistar albino rats (250–300 g; Experimental Animals Breeding and Research Centre, Uludag University, Bursa, Turkey) were used in the present study. Rats were housed under a 12 h light/dark cycle with free access to food and water. The surgical and experimental protocols were approved by the Animal Care and Use Committee of Uludag University and are in accordance with the National Institute of Healths *Guide for the Care and Use of Laboratory Animals* (<http://www.oacu.od.nih.gov/regs/guide/guide1.htm>)

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Surgical and preparative procedures

Rats were anaesthetized with urethane (1.25 g/kg, i.p.) and the left common carotid artery was cannulated with PE50 tubing filled with heparinized saline (250 U/mL). During the arterial cannulation procedure, the vagus nerve and the cervical sympathetic trunk were separated very carefully. For i.c.v. injection of drugs, a burr hole was drilled through the skull 1.5 mm lateral to midline, 1.0 mm posterior to bregma. Then, 22 gauge stainless steel hypodermic tubing was directed through the hole towards the lateral ventricle. The cannula was lowered 4.5 mm below the surface of the skull and was fixed to the skull with acrylic cement. For the i.v. injection of drugs, the left jugular vein of rats was cannulated with polyethylene tubing (PE50) filled with heparinized saline (250 U/mL). The SMA or RA was then exposed by a midline incision and dissected from peripheral tissues. The laser-Doppler flow probe (needle probe; TSD 144; Biopac Systems, Santa Barbara, CA, USA) was secured in a special manipulator stand and placed above the selected artery. In order to reduce artefacts in the perfusion signal that can be caused by the relative movement of tissues induced by breathing, the supported probe was allowed to lightly come into contact with the surface of the artery. The skin of rats was closed to avoid fluid loss due to evaporation throughout the study period. The rectal temperature of rats was maintained at 37–38°C.

Cardiovascular and blood flow recordings

At the end of the preparative procedures, the arterial cannula was connected to a volumetric pressure transducer (BPT 300; Commat, Ankara, Turkey) attached to a DA100B general-purpose transducer amplifier (Commat, Ankara, Turkey). The flow probe was connected to a Biopac LDF 100A Laser Doppler Flow Module. The blood pressure, heart rate and blood flow of rats were recorded and analysed using the MP100 system and Acq-Knowledge software (Biopac Systems). Blood pressure was reported as mean arterial pressure (mmHg) and heart rate was expressed as b.p.m. The laser-Doppler flow meter was designed to measure real-time blood cell perfusion. Laser-Doppler flow signals were recorded as blood perfusion units (BPU). Simultaneously with the BPU output, backscatter signals were recorded to determine whether the probe was working properly. In addition, the backscatter values helped us control whether constant contact between the probe and tissue throughout the measurement.

Haemorrhage and experimental protocol

After connecting the arterial catheter to the transducer and the flow probe to the flow module, baseline blood pressure, heart rate and BPU values of rats were recorded. Rats were allowed to stabilize for 15 min, then acute hypotensive haemorrhage was mimicked by the withdrawal of a total volume of 2.0 or 2.1 mL blood/100 g bodyweight over a period of 20 min. Then, saline or CDP-choline was injected i.v. or i.c.v. and cardiovascular parameters were monitored over the next 90 min. For survival time recordings, rats continued to be monitored until they died. Different sets of animals were used for the measurement of SMA and RA flow in each treatment and dose group to avoid the large surgical incisions, which may affect the outcome of the haemorrhage.

Intracerebroventricular and i.v. injection of drugs

For i.c.v. injections, a 50 µL Hamilton microsyringe was connected to an injection cannula (28 gauge stainless steel tubing), through the polyethylene tubing, and was filled with saline or CDP-choline. The injection cannula was inserted into the guide cannula (22 gauge stainless steel tubing). Drugs, in a volume of 10 µL, were then delivered by hand, slowly, over 40–60 s. At the end of each experiment, the injection site was verified by the injection of 10 µL India ink.

For i.v. injections, the jugular vein was used and drugs were injected through the polyethylene catheter (PE 50) that had been placed before. The injection volume of saline or CDP-choline was 1 mL/kg.

Drugs

Cytidine 5'-diphosphocholine was purchased from Sigma-Aldrich (St Louis, MO, USA). It was dissolved in saline (0.9% NaCl).

Data and statistical analysis

Data are given as the mean ± SEM. Statistical analysis was performed using SPSS for Windows version 11.0 (SPSS, Chicago, IL, USA). For the analysis of blood pressure, heart rate, SMA and RA flow, a two-way mixed-design ANOVA (with independent measures on treatment groups and repeated measures on time periods) was performed with Greenhouse–Geisser adjustment when necessary.¹⁵ Student's unpaired *t*-test and one-way ANOVA with a post hoc Bonferroni *t*-test was used for the comparison of the distributions of the survival time of rats between treatment groups. Two-sided $P < 0.05$ was considered significant.

RESULTS

Effects of haemorrhage on cardiovascular parameters, SMA and RA flow and survival time in anaesthetized rats

The baseline blood pressure and heart rate of anaesthetized rats were 76 ± 1 mmHg and 292 ± 6 b.p.m. ($n = 73$), respectively. Acute haemorrhage decreased blood pressure to 24 ± 1 mmHg ($n = 73$) and increased heart rate to 352 ± 9 b.p.m. Control SMA ($n = 37$) and RA ($n = 36$) flow was 2988 ± 154 and 2161 ± 64 BPU, respectively. At the end of haemorrhage, the flow in these two arteries decreased by approximately two- to threefold. Rats in the saline-treated group survived for 22 ± 2 min ($n = 25$) under these conditions.

Effects of i.c.v. CDP-choline on cardiovascular parameters, SMA and RA blood flow and survival time in haemorrhagic shock

Previous reports have demonstrated the cardiovascular effects of CDP-choline in conscious rats.^{5,6} In the present study, we determined the effect of CDP-choline on the same parameters in anaesthetized rats. The i.c.v. administration of CDP-choline (1.0, 1.5 and 2.0 µmol) produced dose- and time-related increases in blood pressure (Fig. 1a). Analysis of variance revealed a significant effect of dose ($F_{(3,33)} = 9.64$; $P < 0.001$), time ($F_{(3,95)} = 35.33$; $P < 0.001$) and dose–time interaction ($F_{(9,95)} = 5.00$; $P < 0.001$). An approximately 15 and 35 mmHg increase in blood pressures was observed after the injection of 1.0 and 1.5 µmol, i.c.v., CDP-choline. The magnitude of the increase in blood pressure levels was similar after the injection of 1.5 and 2.0 µmol CDP-choline and only partial reversal of hypotension was observed with these two highest doses of CDP-choline. Peak increases were obtained within 5–10 min after the injection of each dose.

Cytidine 5'-diphosphocholine treatment caused a small increase in heart rate in haemorrhaged hypotensive rats (Table 1). Analysis of variance demonstrated that CDP-choline produced a significant time effect on heart rate ($F_{(2,74)} = 26.22$; $P < 0.001$) but the dose ($F_{(3,33)} = 2.14$; $P = 0.11$) and dose–time ($F_{(7,74)} = 0.68$; $P = 0.68$) effects were not significant.

The i.c.v. injection of CDP-choline (1.0, 1.5 and 2.0 µmol) increased the blood flow of the SMA in a dose- and time-dependent manner (Fig. 1b). The decreased blood flow of the SMA returned to prehaemorrhage values within 5–10 min after the injection of 1.5 and 2.0 µmol CDP-choline (Fig. 1b). Analysis of variance

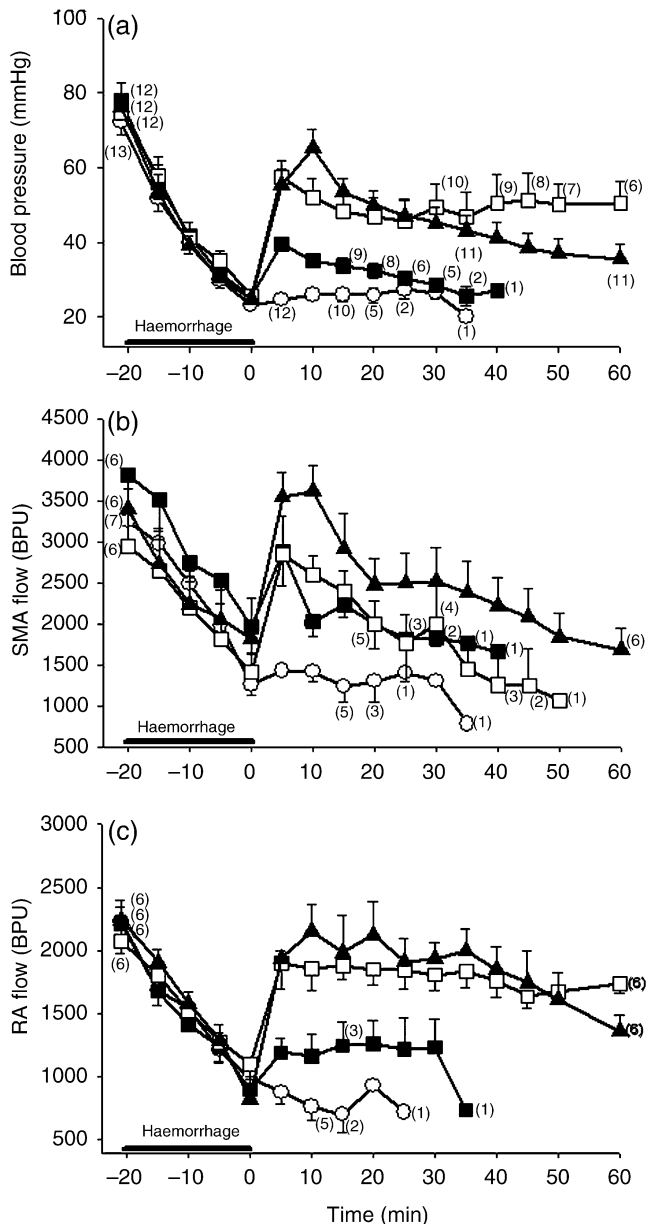


Fig. 1 Effect of i.c.v. cytidine 5'-diphosphocholine (CDP-choline) on (a) blood pressure and the blood flow through the (b) superior mesenteric artery (SMA) and (c) renal artery (RA) in haemorrhaged rats. Rats were subjected to acute haemorrhage and saline (10 μ L; \circ) or CDP-choline (1.0 \blacksquare), 1.5 (\square) and 2.0 μ mol (\blacktriangle) was administered i.c.v. '0' indicates the time at which saline or CDP-choline was injected after haemorrhage. Blood pressure and the flow of arteries were monitored and a 60 min period of observation is shown. The numbers given in parentheses show the number of surviving rats where they differ from the starting number of animals. Data are the mean \pm SEM. Statistical analysis was performed using two-way, mixed-design ANOVA. The ANOVA was not applied after the time at which the number of animals in the saline-treated group was less than five.

confirmed that CDP-choline produced a significant dose ($F_{(3,19)} = 6.20$; $P < 0.01$), time ($F_{(3,57)} = 22.60$; $P < 0.001$) and dose-time interaction ($F_{(9,57)} = 4.30$; $P < 0.001$) on SMA flow.

At 1.0 μ mol, i.c.v., CDP-choline did not significantly change the blood flow of the RA, but complete restoration of decreased

Table 1 Effect of cytidine 5'-diphosphocholine on heart rates of haemorrhaged anaesthetized rats

Treatment	Heart rate (b.p.m.)		
	Before haemorrhage	After haemorrhage	After treatment
Intracerebroventricular injections			
Saline	282 \pm 15	385 \pm 19	412 \pm 11
CDP-choline			
1.0 μ mol	278 \pm 17	393 \pm 17	394 \pm 29
1.5 μ mol	281 \pm 12	322 \pm 25	378 \pm 23
2.0 μ mol	306 \pm 12	347 \pm 16	412 \pm 13
Intravenous injections			
Saline	297 \pm 20	366 \pm 9	369 \pm 13
CDP-choline (250 mg/kg)	278 \pm 10	329 \pm 19	309 \pm 17

Rats were haemorrhaged and saline or cytidine 5'-diphosphocholine (CDP-choline) was injected, i.c.v. or i.v. The heart rate of animals was recorded. The 'after treatment' values represent the heart rate obtained 5 or 10 min after the injections.

Data are the mean \pm SEM of 12–13 rats.

perfusion values of the RA was observed after injection of 1.5 and 2.0 μ mol, i.c.v., CDP-choline (Fig. 1c). Analysis of variance revealed a significant effect of CDP-choline dose ($F_{(3,18)} = 9.77$; $P < 0.001$), time ($F_{(2,36)} = 40.16$; $P < 0.001$) and dose-time interaction ($F_{(6,36)} = 10.87$; $P < 0.001$) on RA perfusion.

In these groups, 1.5 and 2.0 μ mol, i.c.v., CDP-choline increased the survival time of rats from 21 \pm 2 min (saline group) to 95 \pm 16 and 105 \pm 12 min, respectively (Fig. 3a). Analysis of variance confirmed that the effect of CDP-choline on the survival time of rats was significant ($F_{(3,45)} = 5.66$; $P < 0.01$).

Effects of i.v. CDP-choline on cardiovascular parameters, SMA and RA blood flow and survival time in haemorrhagic shock

In anaesthetized rats, the i.v. injection of CDP-choline (250 mg/kg) increased blood pressure by 40% from hypotensive values (Fig. 2a). The pressor effect reached its maximum within 5 min of the administration of CDP-choline and returned to pre-injection values at around 15 min (Fig. 2a). Analysis of variance revealed a significant effect of time ($F_{(2,36)} = 22.65$; $P < 0.001$) and dose-time interaction ($F_{(2,36)} = 17.15$; $P < 0.001$).

Intravenously injected CDP-choline did not significantly affect the heart rate of rats (Table 1).

The i.v. administration of CDP-choline enhanced the blood flow of both SMA and RA (Fig. 2b,c). The effect was transient and flow returned to prehaemorrhage values within 10 min after injection. Analysis of variance confirmed that CDP-choline exerts significant dose ($F_{(1,10)} = 66.44$; $P < 0.001$), time ($F_{(3,30)} = 16.10$; $P < 0.001$) and dose-time interaction ($F_{(3,30)} = 18.09$; $P < 0.001$) effects on the blood flow of the SMA and significant dose ($F_{(1,10)} = 9.01$; $P < 0.05$), time ($F_{(1,10)} = 14.68$; $P < 0.05$) and dose-time interaction ($F_{(1,10)} = 19.47$; $P < 0.05$) effects on RA flow.

The survival time of rats treated with CDP-choline (250 mg/kg, i.v.) was significantly ($P < 0.05$) longer than that of saline-treated rats (50 \pm 10 vs 23 \pm 3 min, respectively; Fig. 3b).

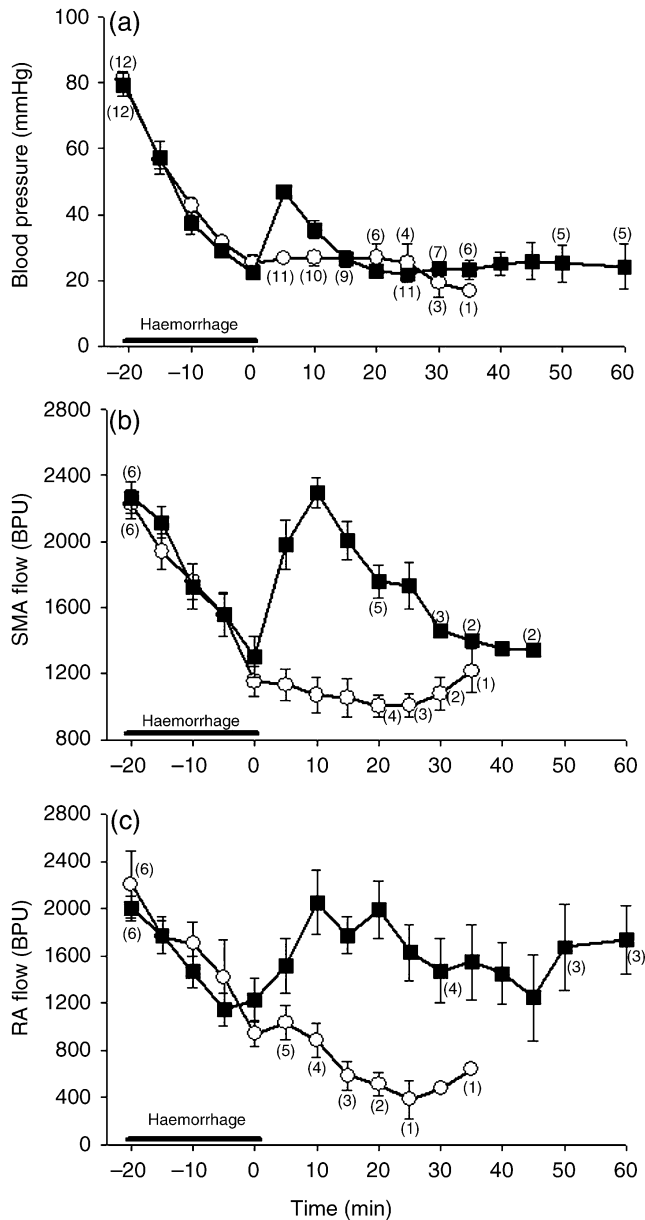


Fig. 2 Effect of i.v. cytidine 5'-diphosphocholine (CDP-choline) on (a) blood pressure and the blood flow through the (b) superior mesenteric artery (SMA) and (c) renal artery (RA) in haemorrhaged rats. Rats were subjected to acute haemorrhage and saline (1 mL/kg; ○) or CDP-choline (250 mg/kg; ■) was injected i.v. '0' indicates the time at which saline or CDP-choline was injected after haemorrhage. Blood pressure and the flow of arteries were monitored and a 60 min period of observation is shown. The numbers given in parentheses show the number of surviving rats where they differ from the starting number of animals. Data are the mean±SEM. Statistical analysis was performed using two-way, mixed-design anova. The anova was not applied after the time at which the number of animals in the saline-treated group was less than five.

DISCUSSION

The present data show that CDP-choline given i.c.v. or i.v. restores the blood flow of SMA and RA and increases the survival time of haemorrhaged hypotensive rats.

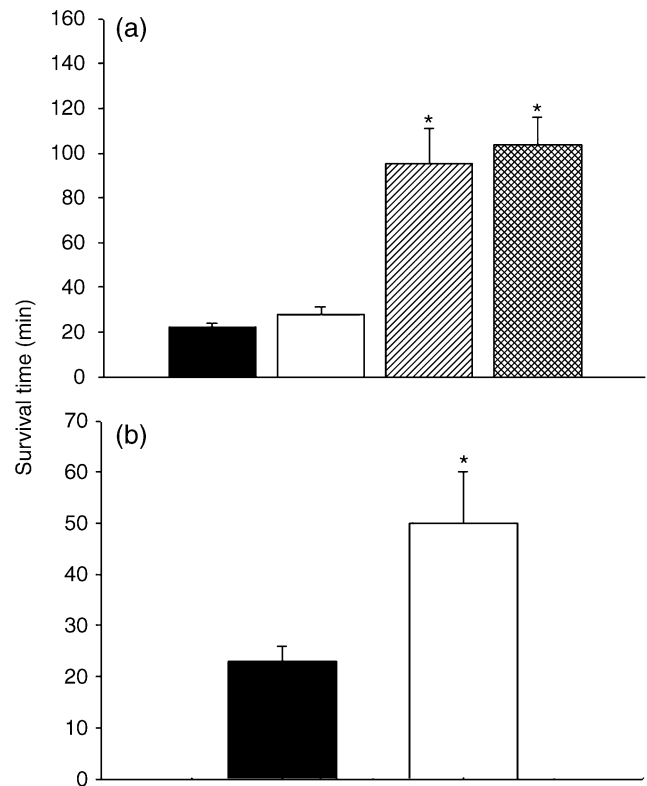


Fig. 3 Survival time of animals injected cytidine 5'-diphosphocholine (CDP-choline) i.c.v. (a) or i.v. (b) in haemorrhaged rats. Rats were haemorrhaged as described in the Methods and saline (10 μL, i.c.v., or 1 mL/kg, i.v.; ■) or CDP-choline (1.0 (□), 1.5 (▨) and 2.0 μmol (▩), i.c.v., or 250 mg/kg (□), i.v.) was administered. All rats were observed until they died and the survival time of rats was recorded. Data are the mean±SEM for six to seven rats. (a) One-way ANOVA with post hoc Bonferroni *t*-test or (b) Student's unpaired *t*-test was used for comparisons of the distribution of survival times between treatment groups. **P* < 0.05 compared with saline.

We have reported previously that CDP-choline injected either way can increase blood pressure and reverse haemorrhagic hypotension through increasing plasma adrenaline and vasopressin levels, but not renin activity.^{5,6} The present study reports, once more, that CDP-choline affects the cardiovascular system positively. Intracerebroventricular or i.v. CDP-choline produced significant increases in blood pressure and partially reversed hypotension in haemorrhaged hypotensive rats. The pressor effect of the drug started immediately; however, it lasted longer in i.c.v.-injected rats than in i.v.-injected rats. These data are in agreement with our previous findings,^{5,6} except for the magnitude of the pressor response to CDP-choline. In the present study, we observed that CDP-choline, injected i.c.v. or i.v. at doses that totally reversed hypotension in our previous study, was not able to completely restore blood pressure. We suggest that the state of consciousness may account for the differences in the findings because, in the present study, we used anaesthetized animals in contrast with the previous study, in which conscious animals were used.

Evidence shows that the splanchnic circulation is particularly sensitive to systemic hypotension and shock conditions.^{12-14,16} Under these conditions, the systemic vasoconstrictor response is disproportionately larger within the mesenteric circulation and persists in spite

of adequate systemic resuscitation.¹³ The perfusion of mesenteric organs is compromised preferentially and the negative impact of the redistribution of blood flow away from the organs served by the mesenteric vasculature can cause detrimental, and even lethal, consequences over the long term. In the present study, we monitored the perfusion values of the SMA because previous reports have indicated that this vessel is more important than the inferior mesenteric artery in maintaining colonic perfusion and mucosal integrity in rats.^{17,18} Acute haemorrhage caused a approximate 60% decrease in the SMA flow of rats. Injection of CDP-choline both i.c.v. and i.v. restored the decreased blood flow of the SMA and completely, but transiently, reversed the hypoperfusion. Conversely, considerable evidence demonstrates that renal blood flow is little affected by mild haemorrhage but is profoundly reduced in severe blood loss. In a previous study, it was reported that bleeding caused a 25% decrease in blood pressure that was accompanied by a 64% decrease in renal blood flow in dogs.¹⁹ In a more recent study, it was reported that experimental haemorrhage that was severe enough to produce 40% blood loss decreased renal blood flow by approximately 80%.²⁰ In agreement with these results, in the present study we observed that renal artery flow values decreased by almost 70–80% immediately after the haemorrhage that produced an approximate 50 mmHg decrease in arterial pressure. Cytidine 5'-diphosphocholine significantly restored the perturbed renal blood flow. These are the first findings showing that CDP-choline restores the flow of important arteries during shock.

Previous studies indicated that the cardiovascular effects of CDP-choline given i.c.v. or i.v. are mediated by the activation of the central cholinergic system^{5,6} because: (i) brain choline levels increased after i.c.v. and i.v. injection of CDP-choline; (ii) peripherally injected choline or cytidine, its metabolic product, failed to exert similar cardiovascular effects; and (iii) blockade of neuronal high-affinity choline uptake or central nicotinic receptors by appropriate pre-treatments abolished the cardiovascular effects of both centrally and peripherally injected CDP-choline.^{5,6} Moreover, in the present study, the observation of incomplete shock reversal obtained following i.v. injection of CDP-choline at a dose much higher than that used with i.c.v. treatment suggests the involvement of central mechanisms in the effect of CDP-choline.

Interestingly, the restoration of SMA and RA blood flow by CDP-choline was not strictly dependent on the level to which blood pressure was restored. In both groups (i.e. i.c.v. and i.v. injection of CDP-choline), CDP-choline completely restored the reduced flow of the SMA and RA after haemorrhage, whereas it increased blood pressure to only 50–60 mmHg (less than prehaemorrhage levels of approximately 80 mmHg) and did not completely reverse hypotension. It has been recently accepted that limited or hypotensive resuscitation may be beneficial in haemorrhagic shock because this strategy could avoid the detrimental effects associated with traditional aggressive resuscitation while still maintaining a level of tissue perfusion. In a recently published study, it was reported that, in rats, an increase of blood pressure to 60 mmHg after resuscitation was enough to restore both systemic and splanchnic perfusion, whereas more aggressive resuscitation to blood pressure \geq 75 mmHg produced greater blood loss and diminished the splanchnic perfusion.¹⁸ The present results are in accord with these previously published data and indicate that CDP-choline may be advantageous in the outcome of hypovolemic shock. The present data about the survival time of rats support this hypothesis, because rats in the

control group survived only approximately 20 min after haemorrhage, whereas CDP-choline-treated rats survived at least two- to fourfold longer depending on the dose administered and the route of injection.

In conclusion, CDP-choline increases SMA and RA flow, together with a partial reversal of hypotension, and prolongs the survival time of anaesthetized rats in haemorrhagic shock.

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REFERENCES

1. Weiss GB. Metabolism and actions of CDP-choline as an endogenous compound and administered exogenously as citicoline. *Life Sci.* 1995; **56**: 637–60.
2. Lopez G-Coviella I, Agut J, Savci V, Ortiz JA, Wurtman RJ. Evidence that 5'-cytidinediphosphocholine can affect brain composition by increasing choline and cytidine plasma levels. *J. Neurochem.* 1995; **65**: 889–94.
3. Adibhatla RM, Hatcher JF, Dempsey RJ. Citicoline: Neuroprotective mechanisms in cerebral ischemia. *J. Neurochem.* 2002; **80**: 12–23.
4. Adibhatla RM, Hatcher JF. Cytidine 5'-diphosphocholine (CDP-choline) in stroke and other CNS disorders. *Neurochem. Res.* 2005; **30**: 15–23.
5. Savci V, Cavun S, Goktalay G, Ulus IH. Cardiovascular effects of intracerebroventricularly injected CDP-choline in normotensive and hypotensive animals: The involvement of cholinergic system. *Naunyn Schmiedeberg's Arch Pharmacol.* 2002; **365**: 388–98.
6. Savci V, Goktalay G, Cansev M, Cavun S, Yilmaz MS, Ulus IH. Intravenously injected CDP-choline increases blood pressure and reverses hypotension in haemorrhagic shock: Effect is mediated by central cholinergic activation. *Eur. J. Pharmacol.* 2003; **468**: 129–39.
7. Lopez G-Coviella I, Agut J, Wurtman RJ. Effect of cytidine (5')-diphosphocholine (CDP-choline) on the total urinary excretion of 3-methoxy-4-hydroxyphenylglycol (MHPG) by rats and humans. *J. Neural Transm.* 1986; **66**: 129–34.
8. Davalos A, Castillo J, Alvarez-Sabin J *et al.* Oral citicoline in acute ischemic stroke: An individual patient data pooling analysis of clinical trials. *Stroke* 2002; **33**: 2850–7.
9. Secher NH, Pawelczyk JA, Ludbrook J. *Blood Loss and Shock*, 1st edn. Oxford University Press, New York. 1994.
10. Hollenberg SM, Kavinsky CJ, Parrillo JE. Cardiogenic shock. *Ann. Intern. Med.* 1999; **131**: 47–59.
11. Anderson WP, Szenasi G. Regional blood flow-renal. In: Secher NH, Pawelczyk JA, Ludbrook J (eds). *Blood Loss and Shock*. Oxford University Press, New York. 1994; Ch. 10.
12. Reilly PM, MacGowan S, Miyachi M, Schiller HJ, Vickers S, Bulkley GB. Mesenteric vasoconstriction in cardiogenic shock in pigs. *Gastroenterology* 1992; **102**: 1968–79.
13. Reilly PM, Bulkley GB. Vasoactive mediators and splanchnic perfusion. *Crit. Care Med.* 1993; **21**: 55–68.
14. Ceppa EP, Fuh KC, Bulkley GB. Mesenteric hemodynamic response to circulatory shock. *Curr. Opin. Crit. Care* 2003; **9**: 127–32.
15. Ludbrook J. Repeated measurements and multiple comparisons in cardiovascular research. *Cardiovasc. Res.* 1994; **28**: 303–11.
16. Varela JE, Cohn SM, Diaz I, Giannotti GD, Proctor KG. Splanchnic perfusion during delayed, hypotensive, or aggressive fluid resuscitation from uncontrolled haemorrhage. *Shock* 2003; **20**: 476–80.

17. Leung FW, Su KC, Pique JM, Thieffn G, Passaro Jr E, Guth PH. Superior mesenteric artery is more important than inferior mesenteric artery in maintaining colonic mucosal perfusion and integrity in rats. *Dig. Dis. Sci.* 1992; **37**: 1329–35.
18. Kozar RA, Holcomb JB, Hassoun HT, Macaitis J, DeSoignie R, Moore FA. Superior mesenteric artery occlusion models shock-induced gut ischemia–reperfusion. *J. Surg. Res.* 2004; **116**: 145–50.
19. Stulak JM, Juncos LA, Haas JA, Romero JC. Systemic hemodynamics and renal function in hemorrhaged dogs resuscitated with cross-linked hemoglobin. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 2000; **278**: R28–33.
20. Kvarstein G, Mirtaheri P, Tonnessen TI. Detection of organ ischemia during hemorrhagic shock. *Acta Anaesthesiol. Scand.* 2003; **47**: 675–86.