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Effects of Ganglionic and Alpha Adrenergic Blocking Agents on the Hemorrhagic Shock in Dogs

Efecto de bloqueadores ganglionares y alfa adrenérgicos sobre el shock hemorrágico en el perro

BRUNO GUNTHER y ENNIO VIVALDI

Departamento de Fisiopatología, Instituto de Ciencias Médico-Biológicas, Universidad de Concepción, Casilla 2407, Concepción, Chile

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A marked increase of the total peripheral resistance (TPR) is one of the outstanding characteristics of the hemorrhagic shock and it is considered that the deleterious outcome of the shock syndrome is due to a deficient oxygen and nutrient supplies to all tissues caused by this maintained arteriolar vasoconstriction. A pharmacological reduction of the TPR should improve the blood flow distribution in the organism and therefore a decreased mortality rate should be expected.

Three different sympathetic blocking agents were injected or infused in anesthetized and heparinized dogs (Dibenamine = 5 mg/kg; Hexamethonium = 2 mg/kg; Phenoxybenzamine = 1 mg/kg, infusion during 30 minutes), which were submitted to a hemorrhagic hypotension during 90 minutes at 45 mm Hg or during 240 minutes at 40 mm Hg.

In almost all treated dogs a decrease was found in the maximal bleeding volume (MBV) in comparison with the control animals. With regard to the automatic reinfusion volume (ARV) and its relationship with the mortality rate, which is commonly observed in the control animals, in the dogs treated with sympathetic blocking agents this correlation is generally absent, because these drugs induced an abnormal high uptake of blood from the reservoir (ARV).

From a theoretical point of view the ganglionic or the alpha receptor blocking agents should have a beneficial therapeutic effect; but in the present shock model the results obtained were not consistent with this hypothesis, since the administration of these drugs after the initiation of hemorrhage did not protect the animals from an irreversible shock.

The principal features of a hemorrhagic shock can be summarized as follows: a reduction of blood volume and of venous return; a low cardiac output correlated with a pronounced increase of total peripheral resistance (TPR).

Both laboratory and clinical studies indicate that vasoconstriction in response to sympathetic system overactivity is an important component of the shock syndrome. Adrenergic blockade has been found to provide a useful procedure to improve tissue blood flow (1,2). As is well known, alpha receptors mediate blood vessel constriction in response to epinephrine and norepinephrine, both circulating and that released from aympathetic nerve endings (3). Phenoxybenzamine and dibenamine are substances which block alpha receptors predominantly and would tend to reduce the amount of peripheral vasoconstriction. On the other hand, ganglionic blockade

such as that induced by hexamethonium interrupts synaptic transmission at all autonomic ganglia, but has no effect on the vascular smooth muscle receptors. Dibenamine, dibenzylene and hexamethonium are known to protect experimental animals from a number of shock procedures (4-11).

Several methods to induce a standardized experimental hemorrhagic shock in anesthesized dogs * have been described previously (12-15). In the present shock model the animals were allowed to bleed into a reservoir at constant pressure (45 mm Hg) and the following variables were measured: initial bleeding volume (IBV), secondary bleeding volume (SBV), maximal bleeding volume (MBV), automatic reinfusion volume (ARV), duration of the hypotensive period (HP) and mortality rate up to 48 hours.

Due to the fact that with the standardized hemorrhagic shock model it is possible to induce either a reversible or an irreversible hemorrhagic shock we are able to assay the effectivity of some therapeutic procedures, which together with the causal treatment and the restitution of the normal blood volume could increase the survival rate of animals which otherwise should die due to an irreversible shock. * Since a pronounced increase of TPR is one of the main features in the evolution towards irreversibility, all drugs which potentially are able to reduce the TPR should eventually decrease the mortality rate in dogs submitted to a standardized irreversible shock (1,4,5).

EXPERIMENTAL PROCEDURE

A standardized hemorrhagic shock was induced in 91 mongrel dogs in accordance with an experimental design * described previously (15). Basically this method is a modification of the Lamson-De Turk (16) reservoir technique with: 1) a constant pressure of 45 mm Hg within the reservoir, and 2) the automatic reinfusion volume (ARV) predetermined in each circumstance.

Furthermore, this experimental procedure allows the induction of a standardized hemorrhagic shock and the assay of ganglionic or adrenergic blocking agents, in order to protect the animals from the deleterious effects of prolonged hypotensive periods (4,8).

With regard to the pharmacological action of a 30 minutes infusion of phenoxybenzamine (1 mg/kg), we have found in control experiments that the maximal arterial hypotension was obtained within the first hour, and that the adrenergic bloking effect was still present 6 hour later.

The 91 animals were submitted to two kinds of experimental procedures.

In accordance with the first protocol, 33 dogs were subdivided into three groups: a) Controls; b) Dibenamine (5 mg/kg i.v.); and c) Hexamethonium (2 mg/kg i.v.). The control animals received an injection of saline, whereas in the other two groups the corresponding drug was administred 30 minutes after the initiation of the bleeding period.

In the second protocol, the 58 animals which were submitted to the standardized hemorrhage, received a constant infusion of 1 mg/kg phenoxybenzamine during 30 minutes, but with a different time schedule: 1) 30 min prior to the initiation of hemorrhage; 2) at zero time; 3) 30 min after the initiation of hemorrhage; 4) 60 min and 5) 120 min after the initiation of hemorrhage. The 16 control animals received the equivalent amount of saline.

In the first group the systemic arterial pressure was maintained at 45 mm Hg during the 90 min of the hypotensive period, while in the second group the hypotensive level was stabilized at 40 mm Hg for 240 min.

The systemic arterial pressure (femoral or brachial arteries) was monitored during the experimental period, and the amount of blood transferred from the animal to the reservoir was determined at the end of the bleeding period (maximal bleeding volume, MBV). Once the MBV was attained spontaneously a certain amount of blood was transferred from the reservoir to the circulatory system and this "uptake" of blood

* Vivaldi, E.: The effect of aureomycin in hemorrhagic shock. Thesis, University of Michigan, 1954.

was measured and designed as the automatic reinfusion volume (ARV). At the end of the predetermined hypotensive period –either 90 or 240 minutes– all the shed blood was transfused into the animal. The mortality rate was determined in accordance with the number of animals which died within the 48 hours after the restitution of the total blood volume.

The statistical significance of the results was evaluated by Student's t-test.

The mortality rate was calculated after van der Waerden (17) where each percentage (p) was estimated in accordance with the following relationship:

$$p = \frac{z + 1}{N + 2} \cdot 100 \qquad (1)$$

z being the number of deaths, and N = number of animal studied.

The standard deviation of a percentage (Sp) was calculated

$$Sp = \sqrt{\frac{p \cdot q}{N + 3}} \qquad (2)$$

where

$q = 100 - p$

Finally, the t-test for two sets of experiments (x, y) was estimated in the following way:

$$t = \frac{P_x - P_y}{\sqrt{Sp_x^2 + Sp_y^2}} \qquad (3)$$

RESULTS

Table 1 summarizes the results obtained for 7 control dogs, in 10 animals infused with dibenamine (5 mg/kg) and in 16 dogs which received 2 mg/kg of hexamethonium.

The maximal bleeding volume (MBV) was expressed as a function of body weight (ml/kg); in the treated dogs the MBV had significantly lower values in comparison with the controls.

With regard to the automatic reinfusion volume (ARV), the dogs which received dibenamine did not show a significant difference with the controls; whereas the ARV of the animals with the hexamethonium infusion was significantly greater than for the other two groups.

On the other hand, the mortality rate is similar in the first two groups (controls and dibenamine) and significantly lower in the hexamethonium treated animals, which have at the same time a reduced MBV and a paradoxically high ARV.

In Table 2 the results obtained in 16 control dogs and in 42 animals receiving phenoxybenzamine (1 mg/kg) are shown. This drug was administered (i.v.) at different moments: 30 minutes prior to hemorrhage; at zero hour, and at 30, 60 and 120 minutes after the initiation of hemorrhage.

TABLE 1

The effect of dibenamine and of hexamethonium injections on the maximal bleeding volume (MBV), the automatic reinfusion volume (ARV) and the mortality rate (o/o) in mongrel dogs submitted to hemorrhagic hypotension (45 mm Hg) during 90 minutes. Each value is the mean ± standard deviation.

Groups	No of Animals	MBV (ml/kg)	ARV (ml/kg)	Mortality Rate (o/o)
Control	7	53.4 ± 10.0	2.9 ± 5.3	78 ± 13.1
Dibenamine (5 mg/kg)	10	39.9 ± 6.6 *	4.9 ± 4.7 NS	58 ± 13.7 NS
Hexamethonium (2 mg/kg)	16	38.1 ± 7.4 *	8.8 ± 4.4 **	27 ± 10.2 *

* Significantly different from controls at $p < 0.005$
** Significantly different from controls at $p < 0.001$
NS = Not significant

TABLE 2

Effect of phenoxybenzamine (1 mg/kg) administered by intravenous infusion during 30 minutes on the maximal bleeding volume (MBV), the automatic reinfusion volume (ARV) and the mortality rate (o/o) in different groups of mongrel dogs submitted to an hemorrhagic hypotension of 40 mm Hg mean systemic pressure during 240 minutes. Each value is the mean ± standard deviation.

Groups	Nº of Animals	MBV (ml/kg)	ARV (ml/kg)	Mortality Rate (o/o)
Controls	16	41.2 ± 6.8	15.2 ± 11.2	90 ± 9.0
Phenoxybenzamine				
a) 30 min. prior to the hemorrhage	18	31.3 ± 7.4 **	0.2 ± 0.6 *	15 ± 7.8 *
b) at zero hour	10	28.4 ± 7.3 *	8.9 ± 9.0 NS	58 ± 13.7 *
c) 30 min. after hemorrhage	6	35.5 ± 7.0 NS	17.3 ± 14.3 NS	62.5 ± 16.1 NS
d) 60 min. after hemorrhage	4	42.5 ± 6.1 NS	27.5 ± 7.0 NS	66.6 ± 17.8 NS
e) 120 min. after hemorrhage	4	38.6 ± 6.7 NS	23.5 ± 16.2 NS	83.3 ± 14.1 NS

* Significantly different from control at $p < 0.005$
** Significantly different from controls at $p < 0.001$
NS = Not significant

The maximal bleeding volume (MBV) was lower in almost all treated animals in comparison with the controls, and significantly lower in dogs which received the drug prior to or at the beginning of hemorrhage.

With regard to the automatic reinfusion volume (ARV), the first group of treated animals had a significantly lower ARV, which correlates with a low MBV and with the lowest mortality rate.

In the animals treated at zero hour and afterwards, the ARV and the mortality rate increase progressively in accordance with the delay in the administration of the drug.

The survival was significantly greater in those animals which received a phenoxybenzamine infusion 30 minutes prior to or at the moment the hemorrhage was initiated (groups *a* and *b*).

DISCUSSION

In a previous paper (15) it was shown that in anesthesized and heparinized dogs which were submitted to a standardized hemorrhagic shock, these animals had a MBV of approximately 50 ml/kg and furthermore than the ARV correlated closely with the mortality rate. For instance if an ARV between 0 and 1 ml/kg was previously established independently of the duration of the hypotensive period, the mortality rate was almost zero. Contrariwise, if the ARV was $\geqslant$ 10 ml/kg the chance of survival was very low (15,18) and the mortality rate close to 90 or 100o/o.

In the above mentioned standardized method (15) the duration of the hypotensive period was not predetermined since only the ARV was established *ad libitum* by the operator and prior to the initiation of the experiment. When a "reversible" shock was expected then an ARV of $\leqslant$ 1 ml/kg was allowed; while an ARV > 8 ml/kg led in almost all animals to an "irreversible" shock. Therefore, when this standardized method was used the ARV was of paramount importance for the evolution of the shock syndrome, since only this parameter was closely correlated with the mortality rate.

In preliminary experiments, the expected correlation between ARV and the

survival rate when the dogs received either dibenamine, hexamethonium or phenoxybenzamine was not observed, and for this reason we had to modify the standardized method in the sense that the hypotensive period should be fixed to 90 or to 240 minutes as shown in Tables 1 and 2.

In all animals which were submitted to one of the sympathetic blocking agents the MBV was significantly lower than in the control animals, with the exception of the phenozybenzamine treated animals, when the administration of the drug was delayed with regard to the beginning of hemorrhage.

In Table 1 we can appreciate that in the dogs infused with dibenamine the ARV was higher than in the control animals, despite the fact that the mortality rate was lower, while in the hexamethonium group the ARV reached 8.8 ml/kg whereas the mortality rate was significantly lower (27o/o). Similar results were obtained with phenoxybenzamine (Table 2). When this drug was administered 30 minutes prior to the beginning of hemorrhage, all variables (MBV, ARV and mortality rate) were significantly lower than the control values. This result seems to indicate that the administration of phenoxybenzamine, when infused 30 minutes prior to hemorrhage, can protect the animals (15o/o mortality rate) in comparison with controls (90o/o mortality rate) confirming the beneficial effects of a pretreatment with this drug (8, 10). In the other four groups (Table 2, b, c, d, e) the MBV is relatively lower than in the control animals, while the ARV is progressively increasing together with the mortality rate. In these cases we see again the correlation between ARV and the mortality rate, but the ARV is always higher in these animals than in the controls. Since phenoxybenzamine was administered in these four groups at zero moment and at 30, 60 and 120 minutes after the initiation of hemorrhage, the ARV was predominantly determined by the vasodilator effect on the arterial and venous vessels due to the increase of the venous capacitance and to a reduction of the TPR of the resistance vessels.

At the present time experiments are being oriented toward studying the effect of acute bleeding on the survival of mongrel dogs. In preliminary experiments we found that an hemorrhage equivalent to 30 or 40 ml/kg was well tolerated and all dogs survived. If we compare these results with those shown in Table 1 and 2 we can appreciate that equivalent values of MBV in the dogs receiving blocking agents are correlated with mortality rate ranging from 15o/o to 83.3o/o, despite the fact that in all these animals the shed blood was reinfused at the end of the experiment. For these reason it is likely that the blocking agents had no beneficial effects when administered after the initiation of hemorrhage.

In an extensive review on cerebral blood flow and brain function during hypotension and shock, Kovách and Sándor (19) concluded that phenoxybenzamine pretreatment prevented: 1) a pressure-dependent decrease in blood flow through the brain at mean arterial pressures of 35 mm Hg; 2) the development of high level of cerebral-tissue CO_2 ; and 3) a "flat" electrocorticographic activity at the same mean pressure level (35 mm Hg).

These authors came to the conclusion that "the protective effect of phenoxybenzamine on cerebral microcirculation and functional impairment in shock suggests the involvement of the sympathetic nervous system and/or catecholamine metabolism in brain damage during hemorrhagic shock. The low flow combined with elevated cellular metabolism produces an imbalance between oxygen delivery and oxygen utilization.

It is noteworthy that the protective effect of phenoxybenzamine (see Table 2) is apparent when this alpha – blocking agent is administered before or just at the beginning of the hemorrhagic period, whereas a delayed treatment has no preventive action. These results can be interpreted in the sense that at the beginning of bleeding a massive discharge of the sympathetic and/or the adrenergic systems are of paramount importance in determining the outcome of shock. If this effect is

prevented by an early administration of the blocking agent, the evolution of the shock syndrome is modified in a favorable manner.

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REFERENCES

1. LEVY, E.Z., NORTH, W.C. and WELLS, J.A. (1954) Modification of traumatic shock by adrenergic blocking agents. *J. Pharmacol. Exper. Therap. 112:* 151-157.
2. THOMPSON, A.E. (1967) Adrenergic blockade in clinical shock of mixed etiology. *In:* Shock and Hypotension. Pathogenesis and Treatment (Mills, L.C., Moyer, J.H., eds.). pp.478-491. Grune and Stratton, New York.
3. THOMPSON, A.E. (1967) Effect of ganglionic and adrenergic blocking agents on regional blood flow. *In:* Shock and Hypotension. Pathogenesis and Treatment (Mills, L.C., Moyer, J.H., eds.). pp. 182-193. Grune and Stratton. New York.
4. REMINGTON, J.W., HAMILTON, W.F., BOYD, Jr. G.H., HAMILTON, W.E. Jr. and CADELL, H.M. (1950) Role of vasoconstriction in the response of the dog to hemorrhage. *Am. J. Physiol. 161:* 116-124.
5. BAEZ, S., SRIKANTIA, S.G., and BURACK, B. (1958) Dibenzamine protecting against shock and preservation of hepatic ferritin systems. *Am. J. Physiol. 192:* 175-180.
6. WATTS, D.T. (1967) Adrenergic mechanisms in hypovolemic shock. *In:* Shock and Hypotension. Pathogenesis and Treatment (Mills, L.C. Moyer, J.H. eds.). pp. 385-391. Grune and Stratton. New York.
7. ANGELAKOS, E.J., BONNER, R.A., IRISH, J.A., ANDREWS, R.A. and BURKETT, D. (1979) Phenoxybenzamine in hemorrhagic shock in the pig. *Circul. Shock. 6:* 198.
8. NICKERSON, M. (1962) Drug therapy of shock. *In:* Shock. Pathogenesis and Therapy. An International Symposium. (Bock, K.D., ed). pp. 356-370. Berlin, Springer.
9. FINE, J. (1962) Comparison of various forms of experimental shock. *In:* Shock. Pathogenesis and Therapy. An International Symposium. (Bock, K.D., ed). pp. 25-39. Berlin, Springer.
10. GÜNTHER, B. and HOLZER, J. (1976) Prevención del shock hemorrágico irreversible mediante el bloqueo de los receptores alfa. *Arch. Biol. Med. Exp. 10:* R-55.
11. HAWTHORNE, E.W. and YSON, E.L. (1967) Effects of sympathetic amines and adrenergic blocking agents on myocardial function. *In:* Shock and Hypotension. Pathogenesis and Treatment (Mills, L.C., Moyer, J.H., eds). pp. 45-68. Grune and Stratton. New York.
12. WALCOTT, W.W. (1945) Standardization of experimental hemorrhagic shock. *Am. J. Physiol. 143:* 254-261.
13. WERLE, J.M., COSEY, R.S. and WIGGERS, C.J. (1942) Observations on hemorrhagic hypotension and hemorrhagic shock. *Am. J. Physion. 143:* 136-401-420.
14. KIRCHHEIM, H. and BAUBKUS, H. (1967) Säure-Base-Veränderungen im standarisierten hämorrhagischen Schock. *Pflügers Arch. 295:* 293-314.
15. VIVALDI, E., MANCINELLI, S. and GÜNTHER, B. (1978) Etiopatogenia del shock irreversible. *Arch. Biol. Med. Exp. 11:* 180.
16. LAMSON, P.D. and DE TURK, W.W. (1945) Studies on shock induced by hemorrhage. XI. A method for the accurate control of blood pressure. *J. Pharmacol. Exper. 83:* 250-252.
17. WAERDEN, B.L. van der (1936) Ueber die richtige Auswertung von Erfolgsstatistiken. *Klin. Wschr. 15:* 1718-1719.
18. JESCH, F., SUNDER-PLASSMANN, L. and MESSMER, K. (1973) Die Bedeutung des uptake im experimentellen hämorrhagischen Schock: Ein Beitrag zur Standarisierung von Schockmodellen. *Res. Exper. Med. 159:* 141-151.
19. KOVÁCH, A.G.B. and SÁNDOR, P. (1976) Cerebral blood flow and brain function during hypotension and shock. *Annu. Rev. Physiol. 39:* 571-596.