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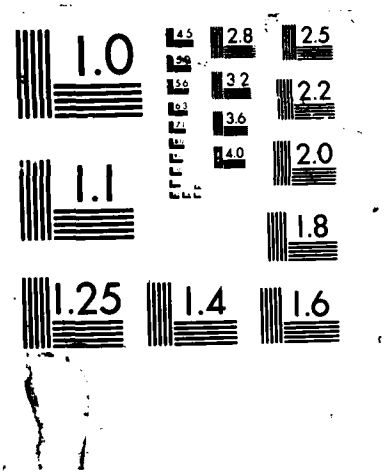
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# Ketanserin Modulates Rabbit Foot Cooling in the Presence or Absence of Exogenous Serotonin

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Running title: Reduced Peripheral Cooling with Ketanserin



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Because hypothermic conditions augment sensitivity to vasoactive amines like seroronin (5-HT) and 5-HT is associated with the etiology of Raynaud's phenomenon, this amine perhaps plays a role in cold-induced vasoconstriction. To determine if 5-HT participated in normal peripheral cooling and if Ketanserin (KET), a 5-HT blocker, modulated such cooling, four groups od New Zealand white rabbits (N=33) were studied. The femoral artery was cannulated to allow perfusion of a hindlimb. A thermister was implated in the foot pad and rectal temperatures were recorded. During cold exposure, 5-HT, KET+5-HT, KET, or saline were perfused through the hindlimb. The rabbit footpad cooled rapidly when exposed to exogenous 5-HT. KET treatment in the presence of exogenous 5-HT was associated with a significantly reduced cooling rate. KET treatment in the absence of exogenous 5-HT was also associated with a significantly elevated limb temperature when compared to controls. This suggested that endogenous 5-HT participated in limbing cooling. Therefore, as noted for Raynaud's disease, 5-HT may also influence peripheral cooling of tissues free of such pathologies.

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## ABSTRACT

Because hypothermic conditions augment sensitivity to vasoactive amines like serotonin (5-HT) and 5-HT is associated with the etiology of Raynaud's phenomenon, this amine perhaps plays a role in cold-induced vasoconstriction. To determine if 5-HT participated in normal peripheral cooling and if Ketanserin (KET), a 5-HT blocker, modulated such cooling, four groups of New Zealand white rabbits (N=33) were studied. The femoral artery was cannulated to allow perfusion of a hindlimb. A thermister was implanted in the footpad and rectum. The hindfoot was exposed to a 15°C bath for 30 min, while footpad and rectal temperatures were recorded. During cold exposure, 5-HT ($5x10^{-2}$ M, group 1), KET (0.1mg/kg)+5-HT (group 2), KET (group 3), or saline (group 4) were perfused through the hindlimb. Groups 2 and 3 were also pretreated with KET (0.1mg/kg perfused over 30 min). The rabbit footpad cooled rapidly when exposed to exogenous 5-HT (group 1). KET treatment in the presence of exogenous 5-HT (group 2) was associated with a significantly ($p<0.05$) reduced cooling rate. KET treatment in the absence of exogenous 5-HT (group 3) was also associated with a significantly ($p<0.05$) elevated limb temperature when compared to controls (group 4). This suggested that endogenous 5-HT participated in limb cooling. Therefore, as noted for Raynaud's disease, 5-HT may also influence peripheral cooling of tissues free of such pathologies. Since Ket treatment did not significantly alter rectal temperature in comparison to controls (39.18±0.95 vs. 38.97±0.97 C°), such treatment with mild cold exposure may have potential in the regulation of peripheral temperature without increasing the risk of hypotherma.

## INTRODUCTION

Under normal physiological conditions, serotonin (5-HT) does not play a major role in local vasomotor control, because circulatory blood levels are below that needed to induce a vascular response (9). However, under conditions in which either 5-HT concentration or receptor sensitivity are augmented, this vasoactive amine can induce constriction of cutaneous blood vessels to disrupt local blood flow (10).

Cooling increases the constriction of vascular smooth muscle induced by 5-HT and other vasoactive substances (3,10,11). Furthermore, ketanserin (Ket), a selective 5-$HT_2$ receptor blocking agent (4,12,13), is beneficial in the treatment of Raynaud's phenomenon (6-8), a disease characterized by an enhanced sensitivity to cold environments. Since Raynaud's disease perhaps represents an example of extreme sensitivity to cold in a continuum of cold sensitivities, a role for 5-HT in the etiology of this phenomenon suggests that 5-HT might also mediate, in part, cold-induced vasoconstriction and enhanced sensitivity to cold, in the absence of such a pathology.

The objective of this study was to identify potential 5-HT mediated events related to peripheral cooling and determine the efficacy of Ket treatment for the reduction of cooling rate of tissue free of diseases such as Raynaud's phenomenon. Serotonin effects were examined during mild cold exposure (15°C) of the rabbit hindfoot. This degree of cooling was selected since it would not likely induce tissue damage and result in platelet activation with the release of additional 5-HT. Our findings indicated that the rabbit footpad cooled more rapidly in response to exogenous 5-HT, since the cooling

response was significantly reduced by Ket treatment. Ket also significantly reduced the cooling rate of the footpad in the absence of exogenous 5-HT. Therefore, normal circulating levels of 5-HT appeared to augment the cooling rate of the rabbit foot. The effects of endogenous 5-HT were modified by a specific blocker for peripheral 5-$HT_2$ receptors. Hence, in the absence of Raynaud's disease, normal circulating levels of 5-HT may influence peripheral cooling and Ket treatment might be of benefit in managing the peripheral response to a cool environment.

## MATERIALS and METHODS

New Zealand white rabbits (N=33) were used. Cannulas were implanted in the femoral artery of the rabbit hindlimb and exteriorized on the dorsal surface of the anesthetized animal. A minimum of 3 days was allowed for recovery from the surgical procedure.

Following recovery, rabbits were anesthetized (xylazine, 5mg/kg and ketamine HCL, 25mg/kg) and maintained under anesthesia during the course of the experiment. A depilatory agent was used to remove hair from the hindfoot and a thermister was implanted in the plantar footpad. A thermocouple was also used to measure rectal temperature. Temperature measurements employed a multipoint temperature scanning system (Leeds and Northrup, North Wales, PA).

The foot with the inserted thermister was covered with a latex sheath and exposed to a bath of 40°C until footpad temperature reached 38°C. Upon obtaining this temperature, tissue distal to the facet of the tibia was

exposed to a 15°C bath for a period of 30 min. Footpad and rectal temperatures were recorded at 30 sec intervals over the 30 min time period. Mean footpad temperatures over 3 min time intervals were calculated and cooling curves generated. During cold exposure, the hindlimb was perfused (0.1 ml/min) with 5-HT ($5x10^{-8}$ M, group 1, N=7), Ket (0.1 mg/kg)+5-HT ( group 2, N=4), Ket (group 3, N=10), or saline (group 4, N=12). In addition, all rabbits receiving Ket perfusions were also pretreated with the drug (0.1 mg/kg perfused over 30 min), just prior to the cold exposure. All other rabbits were pretreated with saline.

Mean footpad temperatures over 3 min time intervals were determined and footpad cooling curves generated. Mean rectal temperatures at the start and end of the cold exposure in the presence or absence of Ket treatment were also calculated.

Footpad cooling curves were compared by analysis of variance and Tukey computation (15). These statistical methods were also used to analyze differences in rectal temperature after cold exposure in the presence or absence of Ket treatment.

## RESULTS and DISCUSSION

Figure 1 illustrates the rabbit footpad cooling curve when the hindlimb was exposed to an exogenous source of 5-HT (group 1). In the presence of exogenous 5-HT, treatment with Ket (group 2) resulted in a significantly ($p<0.05$) elevated cooling curve. Because Ket is a specific blocker of $5\text{-HT}_2$ receptors (4,12,13), this finding suggested that the presence of such receptors

was associated with the rabbit hindlimb vasculature. Moreover, when elevated 5-HT levels (group 1) were present and the limb exposed to a cool environment, footpad cooling was likely mediated, in part, by the reduced peripheral blood flow as a result of 5-HT-induced vasoconstriction. When the effects of exogenous 5-HT were blocked by Ket, footpad cooling was not as rapid (group 2). Such experiments established the appropriateness of this rabbit model for the study of 5-HT-mediated events and confirmed previous findings (4,12,13) which support the ability of Ket to block 5-HT.

In the absence of exogenous 5-HT, Ket treatment (group 3) was also associated with a significantly ($p<0.05$) elevated footpad cooling curve (Fig. 2). As suggested from the findings illustrated in figure 1, the rabbit hindlimb vasculature has receptors for 5-HT, which could be blocked by Ket. Because Ket also reduced the degree of footpad cooling in the absence of exogenous 5-HT, there was the indication that endogenous 5-HT participated in the normal response to cold exposure. It would appear that this response was induced by normal circulating levels of 5-HT, for the cold exposure was relatively mild (15°C) and would not be expected to result in tissue damage (1,2) that might lead to platelet aggregation and additional 5-HT release. Moreover, low temperature reduces platelet aggregation and their release reaction (5). This demonstrated that in addition to peripheral tissue associated with a described pathology like that of Raynaud's phenomenon, the cooling of tissue free of such a disorder may also be mediated, in part, by normal circulating levels of 5-HT.

Reduced blood flow to peripheral areas is one mechanism to ensure maintenance of core body heat during cold exposure. Pharmacological

approaches to improve peripheral blood flow in cold environments may hasten the development of hypothermia. A statistical analysis of rectal temperature at the start and end of the 30 min cold exposure in the presence or absence of Ket treatment (groups 3 and 4) did not reveal any significant differences (Table 1). Apparently, such a mild cold exposure induced significant peripheral cooling without a significant reduction in rectal temperature. Moreover, Ket treatment reduced the noted peripheral cooling without a further decrease in core body heat. Thus, under certain environmental conditions (e.g. mild cold exposure for short time periods) such pharmacologically oriented management of peripheral cooling may be warranted without significantly increasing the risk of hypothermia, above the risk associated with the absence of treatment to reduce peripheral cooling.

In summary, Ket treatment reduced the limb cooling rate associated with exogenous 5-HT. In the absence of exogenous 5-HT and under conditions (15°C) which should not increase 5-HT release from platelets, Ket treatment was associated with no further enhancement of body heat loss and a significantly reduced degree of limb cooling. It would appear that normal circulating levels of 5-HT participated in rabbit limb cooling and that blocking the effects of this amine may serve as one element in the regulation of peripheral temperature in the response to mild cold exposure. Moreover, as noted with Ket treatment following forstbite injury (14), such treatment may also have potential in frostbite prevention.

## REFERENCES


1. Bourne, M.H., M.W. Piepkorn, F. Clayton, and L.G. Leonard. Analysis of microvascular changes in frostbite injury. J. Surgical Res. 40, 26-35 (1986).

2. Bowers, W.D., R.W. Hubbard, R.C. Daum, P. Ashbaugh, and E. Nilson. Ultrastructural studies of muscle cells and vascular endothelium immediately after freeze-thaw injury. Cryobiology 10, 9-21 (1973).

3. Lindblad, L.E., J.T. Shepherd, and P.M. Vanhoutte. Cooling augments platelet-induced contraction of peripheral arteries of the dog. Proc. Soc. Exp. Biol. Med. 176, 119-112 (1984).

4. Lysen, J.E., F. Awonters, L. Kennis, P.M. Laduron, J. Vanderberk, and P.A.J. Janssen. Receptor binding profile of R41,468, a novel antagonist at 5-$HT_2$ receptors. Life Sci. 28, 1015-1022 (1981).

5. Newhouse, P. and C. Clark. The variability of platelet aggregation. In "Platelet Function" (D.A. Triplett, Ed.), pp 63-107. Amer. Soc. Clin. Path., Chicago, IL (1978).

6. Roald, O. and E. Siem. Treatment of Raynaud's phenomenon with ketanserin in patients with connective tissue disorders. Brit. Med. J. 289, 577-579 (1984).

7. Seibold, J.R. and A.H.M. Jageneau. Treatment of Raynaud's phenomenon with ketanserin, a selective antagonist of the serotonin$_2$ (5-HT$_2$) receptor. Arthritis and Rheumatism 27, 139-146 (1984).

8. Stranden, E., O.K. Roald, and K. Krohg. Treatment of Raynaud's phenomenon with the 5-HT$_2$ receptor antagonist ketanserin. Brit. Med. J. 285, 1069-1071 (1982).

9. Vanhoutte, P.M. Heterogeneity in vascular smooth muscle. In "Microcirculation" (G. Kaley and B.M. Altura, Eds.), Vol. 2, p. 181-309. University Park Press, Baltimore, MD, 1978.

10. Vanhoutte, P.M. Physical factors of regulation. In "Handbook of Physiology" (D.F. Bohr, Ed.), Vol. 2 p.447. Williams and Wilkins, Baltimore, MD, 1980.

11. Vanhoutte, P.M. and W.J. Janssens. Local control of venous function. Microvascular Res. 16,196-214 (1978).

12. Van Nueten, J.M., P.A. Janssen, J. Van Beek, R. Xhoneux, T.J. Verbeuren, and P.M. Vanhoutte. Vascular effects of ketanserin (R 41 468), a novel antagonist of 5-HT$_2$ serotonergic receptors. J. Pharmacol. Exp. Ther. 288, 217-230 (1981).

13. Van Nueten, J.M., J.E. Leysen, J.A.J. Schuurkes, and P.M. Vanhoutte. Ketanserin: A selective antagonist of 5-HT$_2$ serotonergic receptors. Lancet-I, 297-298 (1983).

14. Vayssairat, M., P. Priollet, A. Hagege, and E. Housset. Does ketanserin relieve frostbite? Practitioner 230, 406-408 (1986).

15. Zar, J.H. "Biostatistical Analysis", pp. 185-205. Prentice-Hall, Inc., Englewood Cliffs, NJ, 1984.

Figure 1. Effect of exogenous serotonin (5-HT) on rabbit footpad cooling at 15°C in the presence or absence of ketanserin (Ket) treatment. Points are mean footpad temperatures (determined over 3 min intervals) with their standard error values.

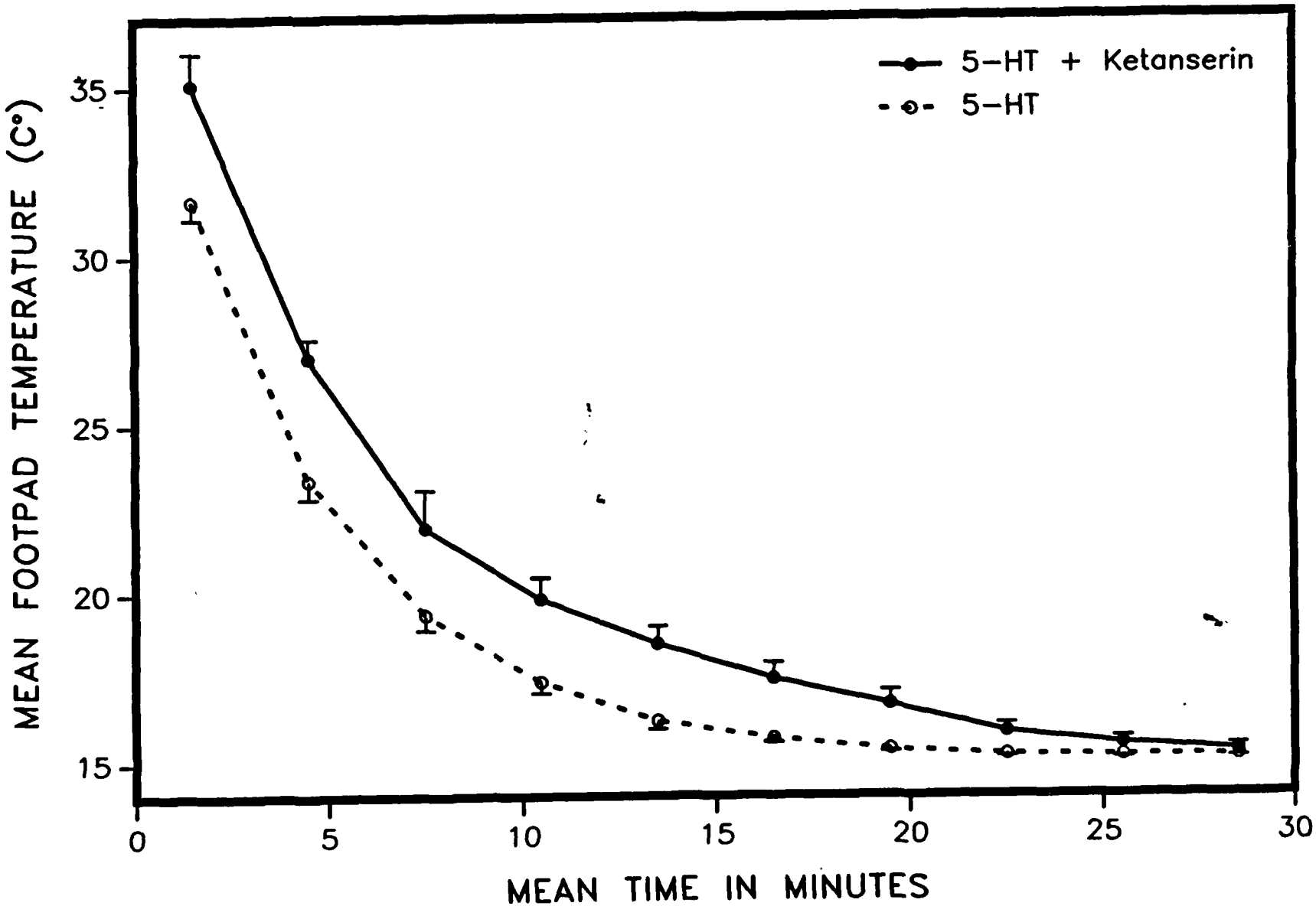
5-HT + Ketanserin
5-HT
MEAN FOOTPAD TEMPERATURE (C°)
35
30
25
20
15
0
5
10
15
20
25
30
MEAN TIME IN MINUTES

Figure 2. Effect on rabbit footpad cooling at 15°C in the presence or absence of ketanserin (Ket) treatment. Points are mean footpad temperatures (determined over 3 min intervals) with their standard error values.

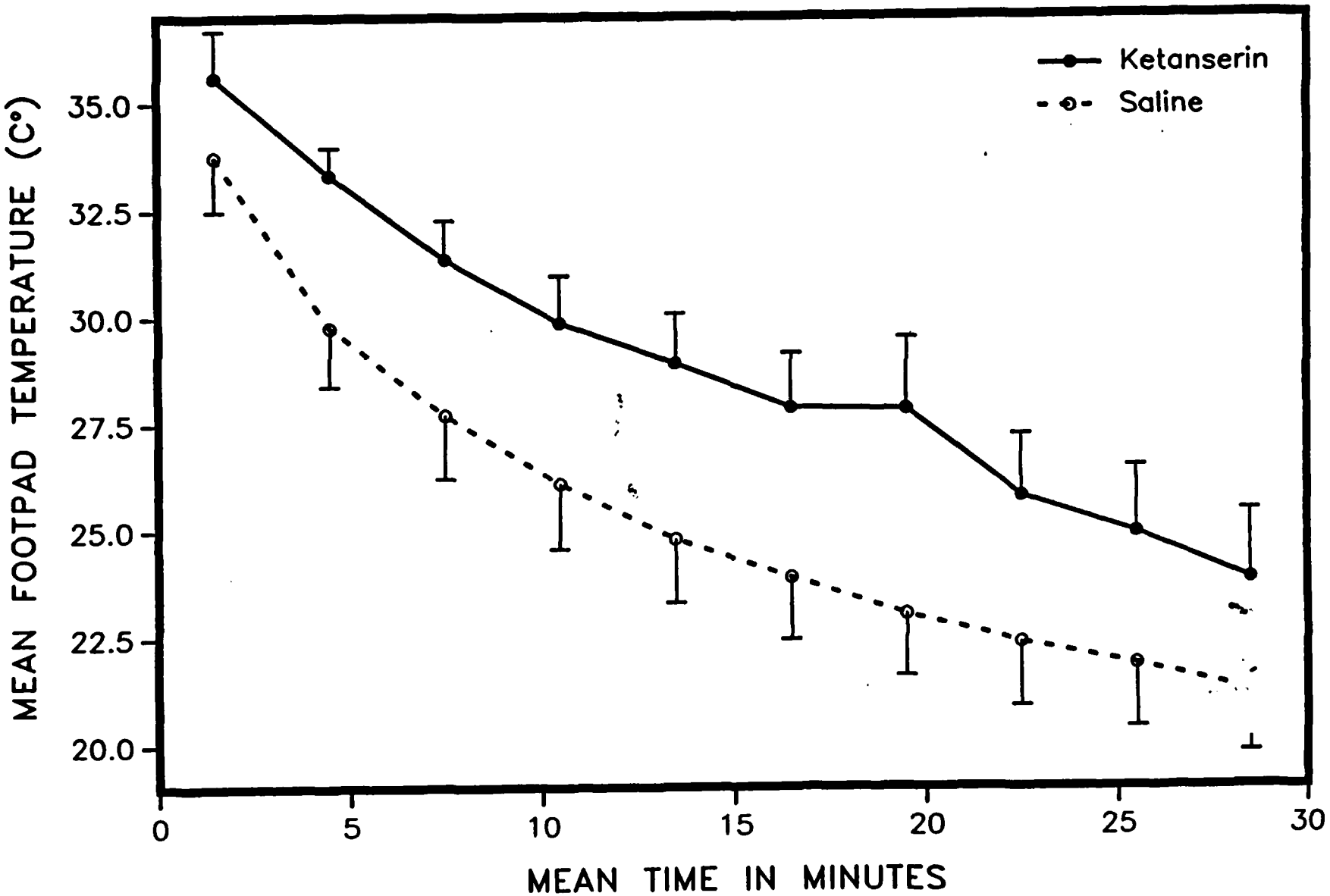
Ketanserin
Saline
MEAN FOOTPAD TEMPERATURE (C°)
35.0
32.5
30.0
27.5
25.0
22.5
20.0
0
5
10
15
20
25
30
MEAN TIME IN MINUTES

Table 1. Effect of ketanserin treatment and footpad cooling (15°C) on rabbit rectal temperature.

| | Rectal Temperature (C°) at start and end of cold exposure | |
|---|---|---|
| Substance Perfused | Start | End |
| Saline | 39.97* ±0.61 | 38.97 ±1.02 |
| Ketanserin | 40.03 ±0.68 | 39.18 ±0.95 |

*values are means ± standard deviation

## List of Figures

## Disclaimer

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