THE EFFECT OF OXYTOCIN ON BLOOD VESSELS OF CREMASTER MUSCLE OF THE RAT

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Oxytocin administered intravenously to the anaesthetized rat produced dilatation of the blood vessels of the cremaster muscle at concentrations ranging from 2.5×10^{-11} to 2.5×10^{-9} M. When applied topically to the exposed vessels it produced constriction at concentrations ranging from 2.5×10^{-12} to 5.0×10^{-8} M. Oxytocin was thus similar to adrenaline in eliciting opposite effects when applied to the serosa or to the intima of skeletal muscle blood vessels.

Introduction It is known from clinical observation that administration of exogenous oxytocin to women can cause flushing of the skin and a measurable fall in blood pressure. Pickford and her colleagues (Pickford, 1966) showed that in man this response occurred in skin and muscle vessels of the limbs, and that it could be converted to vasoconstriction by sympathectomy or by the presence of circulating ovarian hormones. Using male rats Honoré & Lloyd (1961) observed dilatation of the vessels of the mesoappendix in response to intravenous injection of 75 mu doses of oxytocin; but Berde (1965) was unable to record any difference from controls in the ^{86}Rb content of diaphragm and gastrocnemius muscles after infusion of oxytocin, i.e., he found no increase in blood flow after oxytocin. It was considered that Grant's (1964) technique of observing the blood vessels of rat cremaster muscle under the dissecting microscope might reveal more directly how the muscle blood vessels respond to oxytocin. Grant was satisfied that, in respect of all the substances which he used, the vessels in the cremaster muscle responded in the same way as blood vessels of the musculature of the thigh, and he considered that cremaster vessel responses might be taken to be representative of responses of 'limb skeletal muscles in general'.

Methods Young male rats (170-200 g) were anaesthetized with urethane, the jugular vein was cannulated, and a slow infusion of gelatin-Ringer was initiated at a rate of about 0.5 ml/h; the cremaster muscle was exposed and irrigated with gelatin-Ringer at 30-31° C for examination of the blood vessels under the dissecting microscope as described by Grant (1964). When the effect on the blood vessels of intravenous administration of hormones or drugs was to be examined, the material was injected into the venous cannula. When the effect of topical application was to be examined, the material was added to the pool of gelatin-Ringer irrigating and bathing the cremaster muscle, the volume of this pool being estimated to be 0.5 ml. The effect of each dose treatment was examined in at least five animals. The validity of the method was confirmed by repeating Grant's observations on the effects of histamine, adrenaline, noradrenaline and acetylcholine. In all experiments the sensitivity of the preparation was confirmed by observing the dilatation of the blood vessels produced by a small intravenous dose of adrenaline (usually 5 μg/rat). Solutions for injection or irrigation were prepared in gelatin-Ringer. Oxytocin was Pitocin (Parke Davis) having oxytocic activity 10 u/ml and containing chlorbutol 0.5%.

Results Intravenous administration of oxytocin in doses ranging from 0.1 mu to 10 mu always produced dilatation in the cremaster muscle blood vessels, with onset about 1 min (range 0.5 to 3 min) after injection. This dilatation was recorded as vessels becoming 'clearer', 'more distinct', 'redder' or 'wavy'. Branches appeared on the large vessels, and small vessels opened up and became visible where they had been previously constricted and invisible. Both arterial and venous vessels became dilated. Usually a clear dilatation was still visible at 5 to 10 min and thereafter a slow recovery took place, and the normal resting appearance was regained at about 15 minutes. Occasionally, along with the general dilatation, or following it, constriction was observed in single arterial branches. When injections were repeated the recovery from dilatation took place more quickly, and in some animals after repeated injections the response to the smallest dose could no longer be detected. Table 1a shows the doses used. Assuming that injected material would be dispersed in a plasma volume of about 10 ml, the concentration of oxytocin reaching the intima of the blood vessels was calculated and is shown in Table 1a. Dilatation of the vessels was produced by concentrations of oxytocin down to 2.5×10^{-11} M. Since rat pituitaries were found on

Table 1 Effect of drugs on small blood vessels of rat

Vascular bed	*Surface exposed to drug*	*Treatment*	*Estimated concentration of drug (M) applied to blood vessels*	*Observed effect*
(a) Cremaster muscle	Intima	*Oxytocin*		
		Intravenous 0.1 mu	2.5×10^{-11}	D
		Intravenous 1.0 mu	2.5×10^{-10}	D
		Intravenous 4.0 mu	1.0×10^{-9}	D
		Intravenous 10.0 mu	2.5×10^{-9}	D
(b) Cremaster muscle	Serosa	*Oxytocin*		
		Irrigation of tissue 1.0 μu/ml	2.5×10^{-12}	C
		Irrigation of tissue 10.0 μu/ml	2.5×10^{-11}	C
		0.1 mu added to 0.5 ml pool	5.0×10^{-10}	C
		1.0 mu added to 0.5 ml pool	5.0×10^{-9}	C
		10.0 mu added to 0.5 ml pool	5.0×10^{-8}	C
(c) Mesoappendix	Intima	*Oxytocin* (Lloyd, 1959)		
		Intravenous 10-30 mu	10^{-9}-10^{-8}	D
(d) Mesoappendix	Serosa	*Oxytocin* (Lloyd, 1959)		
		Bathed with 50 mu/ml	1.0×10^{-7}	D
(e) Cremaster muscle	Intima	*Adrenaline* (Grant, 1964)		
		Intravenous 0.01 to 0.1 μg	10^{-9}-10^{-8}	D
(f) Cremaster muscle	Serosa	*Adrenaline* (Grant, 1964)		
		Local application 0.01-10.0 ng/ml	10^{-11}-10^{-7}	C

D = dilatation, C = constriction
Sections (a) and (b) are the results of the present investigation; sections (c) and (d) are from Lloyd (1959); sections (e) and (f) are from Grant (1964).

assay to have approximately 500 mu of oxytocic activity, it could be calculated that the smallest effective dose of oxytocin was equivalent in terms of oxytocic activity to the addition to the circulating plasma of about 1/5000 of a rat pituitary. Control injections were made with equivalent volumes of gelatin-Ringer, and gelatin-Ringer with chlorbutol (1.0 μg and 5.0 μg), and none of these injections produced any detectable effect on the blood vessels.

Topical application of oxytocin in doses ranging from 0.5 μu to 10 mu added to the 0.5 ml pool of gelatin-Ringer bathing the cremaster muscle always produced vasoconstriction of the blood vessels. The lower doses of oxytocin were applied by having a double irrigation system, and alternating between irrigation with gelatin-Ringer and irrigation with gelatin-Ringer containing oxytocin. Onset of vasoconstriction was seen only 1 to 1.5 min after the irrigation with oxytocin had been started, and if the irrigation was continued the vasoconstriction passed off after about 5 to 10 minutes. When irrigation with oxytocin had produced an obvious vasoconstriction, then changing to gelatin-Ringer hastened the recovery to normal. The higher doses of oxytocin were applied by gently adding the oxytocin, suitably diluted in gelatin-Ringer, to the pool of gelatin-Ringer surrounding the muscle. Onset of vasoconstriction was usually recorded at about 1 minute. It was frequently noted that constriction would begin at one end of a large vein, and would slowly spread along the visible length of it; irrigation with gelatin-Ringer continuing, the vasoconstriction began to diminish after about 5 min, and the appearance returned to normal at about 10 minutes. Table 1b shows the doses and concentrations of oxytocin which produced vasoconstriction on topical application.

Discussion It is notable that in the present experiments very small intravenous doses of oxytocin, producing plasma concentrations of approximately 2.5×10^{-11} M, have been shown to dilate the blood vessels of the rat cremaster muscle. The possible dilator effect of the chlorbutol, also present in the solutions, must be considered, as has been discussed by Somlyo, Woo & Somlyo (1966). The highest dose of oxytocin used (10.0 mu) would be accompanied by 5.0 μg of chlorbutol. Control injections showed that this amount of chlorbutol never produced vasodilata-

tion; and when diluted in the plasma it would give a concentration of approximately 0.5 μg/ml which is far below the threshold concentration of 0.1 mg/ml which Somlyo *et al.* (1966) recorded for arterial smooth muscle. The possibility of the observed dilatation being due to chlorbutol is thus ruled out. Lloyd (1959) observed that intravenous injection of oxytocin produced dilatation in the vessels of the rat mesoappendix, the threshold dose being 10 mu/rat (Table 1c). This concentration was of a different order from that in the human experiments of Deis, Kitchin & Pickford (1963) where intravenous injection of 500 mu oxytocin was shown to produce increased hand blood flow, and the concentration of oxytocin reaching the microvasculature would have been approximately 2.5×10^{-10} M. If we accept Grant's (1964) demonstration that the response of the cremaster muscle vessels is characteristic of skeletal muscle vessels in general, then the present results might be taken to indicate that in the rat, just as in man, skeletal muscle vessels are dilated by oxytocin in concentrations of the order of 10^{-10} M.

Topical application of oxytocin to the exposed tissue of the cremaster muscle produced results which were different from those seen by Lloyd (1959) in the mesoappendix. Lloyd observed dilatation (Table 1d) whereas in the present experiments (Table 1b) vasoconstriction was always seen. The present findings agree with the observations of Altura (1972); in his experiments oxytocin topically applied produced constriction in the metarterioles of rat mesentery, with a threshold concentration of 10^{-8} M and a dose-dependent increase to a maximum at 5×10^{-5} M.

The observed difference in the response to oxytocin according to whether it is applied to the intima or to the serosa of the microvasculature is reminiscent of Grant's (1964) observations on adrenaline (Table 1e and 1f). The difference might derive, as has been suggested by Altura & Hershey (1967) from the different responses of arterioles, venules and capillaries, or perhaps, as suggested by Grant with reference to adrenaline, from the anatomical situation of the receptors in the smooth muscle of the vessels. The only previous indication of such a difference in respect of oxytocin seems to be in the work of Altura & Hershey (1967) who noted that topically applied oxytocin constricted the metarterioles of rat mesentery, while if intravenously administered it depressed the contractile response to catecholamines.

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