Inefficiency of Isoprenaline to Induce Drinking in the Goat

By

KERSTIN OLSSON and MATS RUNDGREN

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Abstract


Isoprenaline, which acts as a potent dipsogen in water-satiated rats and dogs, did not elicit water intake when infused intravenously at 0.1 or 0.3 μg/kg min⁻¹ in non-hydrated goats. Even the low dose of the drug caused a marked reduction of parotid salivary flow. The possibility is discussed that reduced salivary secretion might be the particular effect which makes isoprenaline dipsogenic in prandially drinking species. The intravenous infusion of isoprenaline at the high dose level caused an inhibition of the water diuresis of hydrated goats, concomitant with reduced renal Na⁺ excretion and a marked, sustained fall in arterial blood pressure. Significant amounts of ADH were recovered from the urine secreted during the antidiuresis. This ADH-release was apparently not due to central β-adrenergic stimulation since no inhibition of the water diuresis was observed during intraventricular infusions of isoprenaline. Rather, the ADH-release appears to have been secondary to the isoprenaline-induced fall in arterial blood pressure.

Both a marked dipsogenic and an antidiuretic effect of β-adrenergic agonists have been demonstrated in two mammalian species, the rat (Zamboni and Siro-Brigiani 1966, Lehr, Mallow and Krukowski 1967) and the dog (Fitzsimons and Szczepanska-Sadowska 1974). From available data, it appears that the mechanisms behind this β-adrenergic effect on the water turnover differ in the two species.

Suggestive evidence has been presented that the ultimate cause of β-adrenergic drinking in the rat is an activation of the renin-angiotensin system, previously shown to induce drinking in this species (cf. FITZSIMONS 1972). Hence, the administration of β-adrenergic agonists to rats causes an elevation of plasma renin activity (Peskar et al. 1970), and rats no longer drink in response to β-adrenergic agonists after nephrectomy (Houpt and Epstein 1971).

An activation of the renin-angiotensin system appears to be of no, or of only minor importance for the dipsogenic response to β-adrenergic agonists in the dog. Angiotensin II is relatively ineffective in eliciting drinking in this species (Kozlowski, Drzewiecki and Żurawski 1972) and nephrectomy does not significantly reduce drinking in response to isoprenaline, which suggests that non-hormonal mechanisms are much more important for the dipsogenic response than in the rat (Fitzsimons and Szczepanska-Sadowska 1974). The dog is a prandial drinker (Wolf 1958), but it remains to be studied whether oropharyngeal factors contribute to its isoprenaline induced drinking.
The apparent difference between the mechanisms behind β-adrenergic drinking in the rat and in the dog has provided the incitament for this study of the effects of isoprenaline on the water balance and the salivary secretion in the goat.

**Methods**

*Animals.* 10 adult female goats (b.wt. 35-40 kg) were used. The animals were routinely kept in metabolism cages where all experiments were conducted. They were fed chopped hay every morning at 8 o'clock and the experiments were not started until at least 2 h later. They had free access to water at a temperature of 20 ± 1°C and were maintained in positive sodium balance by obtaining 6 g of NaCl in 300 g of commercial grain mix each afternoon.

**Administration of isoprenaline.** Isoprenaline, dissolved in isotonic saline was infused intravenously via a polyethylene cannula introduced into the jugular vein. In most experiments isoprenaline hydrochloride (Isuprel, Winthrop) was used. Isoprenaline sulphate (Aludrin, Boehringer Sohn) was infused in a few experiments. The dose of isoprenaline was 0.1 or 0.3 μg/kg min⁻¹ and the infusion periods were 50 or 60 min.

Infusions (20 μl/min) into the cerebrospinal fluid (CSF) of the lateral cerebral ventricle were performed in two of the goats via a permanently implanted three-cannula system as earlier described (Åkerlund, Andersson and Olsson 1973). Isoprenaline hydrochloride, dissolved in isotonic saline, was infused into the ventricle at doses of 0.5 or 1 ng/kg min⁻¹. In some experiments, angiotensin II (Hypertensin, Ciba), dissolved in 0.5 M NaCl, was infused into the CSF of the lateral ventricle subsequent to the intraventricular isoprenaline infusion. The dosage of angiotensin II was 0.8 ng/kg min⁻¹.

**Blood pressure and heart rate recordings.** Four of the goats had a polyvinyl catheter implanted via the superficial temporal artery into the carotid artery as described earlier (Eriksson, Fernández and Olsson 1971). The free end of the catheter was attached to one horn of the goat and the catheter was flushed daily with heparin solution. During the experiments the polyvinyl catheter was connected to a Statham pressure transducer and the systolic/diastolic blood pressures were recorded on an ink-writing polygraph. When not registered on the polygraph, the heart rate was determined at intervals by auscultation.

**Collection of saliva.** One of the goats was prepared with a polyvinyl catheter (O.D. 2.0 mm, I.D. 1.4 mm) permanently implanted into the parotid duct (cf. Hecker 1974). The free end of the catheter was passed through the skin of the cheek and then connected to a stainless steel barrel. The barrel, which was provided with an external and an internal flange, was pushed through the skin opposite to the second premolar tooth. In this manner, the saliva was returned to the animal between experiments. During experiments the free end of the catheter was disconnected from the barrel and the saliva was collected in graded glass tubes.

**Hydration.** A water diuresis was established by giving the goats, by stomach tube, 100 ml/kg b.wt. of 38°C water ½ to 2 h before the isoprenaline infusions were started.

**Analyses.** Urine was collected in 10 min samples via a retention catheter inserted into the urinary bladder. Urine and salivary Na⁺ was determined by use of an EEL flame photometer and an “Advanced osmometer Inc.” was used for determinations of the osmolality of these fluids and the blood plasma. The mean plasma osmolality was found to be 290 mosm/kg during hydration. Therefore this value was used for calculations of renal free water clearance (CH₂O) in the experiments performed in hydrated goats.

**Assay of antidiuretic hormone (ADH) in the urine.** The method described by Frandsen (1969) was employed with some modification for separation of ADH from urine, and the amount of hormone present was assayed in the hydrated, nonanesthetized goat (Lishajko 1975). This proceeding has been found to give approximately 10 per cent recovery in the urine of arginine vasopressin injected intravenously in the goat.

**Results**

**Intravenous infusions**

**Tests for effect on water intake.** 8 non-hydrated goats were subjected to 60 min intravenous infusions of isoprenaline. In 7 expts. the low dose (0.1 μg/kg min⁻¹) was administered, whereas the high dose (0.3 μg/kg min⁻¹) was given in 5 expts. During these infusions the
animals did not show any interest in the water available in front of them, but continued to eat hay now and again.

Renal effects. 6 pre-hydrated goats were subjected to infusions of the low dose of isoprenaline (7 expts.). As shown in Fig. 1 (circles) these infusions did not cause any obvious change in the positive renal $C_{H_2O}$ or in the renal $Na^+$ excretion. However, when the high dose of isoprenaline was infused in 4 pre-hydrated animals (7 expts.) a marked drop in urine flow was observed within the first 5 min of the infusion. However, renal $C_{H_2O}$ did not become negative until 30 min after the onset of the isoprenaline infusion (Fig. 1, dots). Concomitant with the antidiuresis there was a marked fall in renal $Na^+$ excretion. In two of the expts. the urine was collected before, and during the entire periods of negative $C_{H_2O}$ for separation of ADH. Subsequent biological assay revealed the presence of roughly 5 m U of ADH in the urine collected during each of the periods of antidiuresis, whereas no ADH was recovered in the preceding urine samples. With an estimated recovery of 10 per cent, it indicates that the 60 min intravenous infusion of the high dose of isoprenaline caused a release of approximately 50 mU of ADH.

Effects on heart rate and arterial blood pressure. All intravenous isoprenaline infusions induced tachycardia within few minutes. During infusions of the low dose of isoprenaline, the heart rate increased from about 90 beats/min to nearly 200 beats/min (Fig. 2), whereas the heart rate was 200 to 250 beats/min during infusions of the high dose of the drug. There was a moderate fall in blood pressure during the initial 20 min of the infusion of the low dose of isoprenaline. Then the blood pressure gradually retained pre-infusion values (Fig. 1,
Fig. 2. Marked decrease in the unilateral parotid salivary flow during intravenous infusions of isoprenaline at 0.1 µg/kg min⁻¹. Note the marked increase in heart rate. Number of experiments: 4 (mean and S.E.).

circles). A much more marked and sustained hypotension was observed during the intravenous infusions of the high isoprenaline dose (Fig. 1, dots).

Effects on parotid salivary flow. The secretion from one of the parotid glands was registered during 4 intravenous infusions of isoprenaline in one goat. Isoprenaline was infused intravenously for 50 min at 0.1 µg/kg min⁻¹. Within 20 min of infusion the salivary flow had decreased from 1.1 ± 0.2 ml/min (mean and S.E.) to 0.6 ± 0.1 ml/min. At the end of the infusion period the flow rate was reduced to 0.5 ± 0.1 ml/min. After cessation of the infusion the salivary flow slowly returned to pre-infusion level (Fig. 2).

Intraventricular infusions
Two non-hydrated goats were used for infusions of isoprenaline into the CSF of the lateral ventricle. The dose of isoprenaline was 0.5 ng/kg min⁻¹ (5 expts.) and 1 ng/kg min⁻¹ (2 expts.).

Fig. 3. Lack of effects on water intake and renal sodium excretion during an isoprenaline infusion into the cerebrospinal fluid of the lateral ventricle. Note the marked cumulative drinking and the natriuresis in response to the subsequent intraventricular infusion of angiotensin II dissolved in 0.5 M NaCl. Dose of isoprenaline: 1 ng/kg min⁻¹. Dose of angiotensin 0.8 ng/kg min⁻¹. Rate of infusion: 20 µl/min.
The infusion periods were 30 or 50 min. None of these infusions induced drinking or affected renal sodium excretion. As expected (cf. Andersson and Olsson 1973) however, the animals responded to subsequent intraventricular infusions of angiotensin II in hypertonic NaCl with cumulative drinking and natriuresis (Fig. 3). Two 50 min intraventricular infusions of isoprenaline (0.5 and 1 ng/kg min⁻¹) were also performed when the goats were hydrated. The renal C₃H₄O remained positive throughout these infusion periods.

No tachycardia was observed during any of the intraventricular infusions of isoprenaline.

Discussion

Systematically administered, β-adrenergic agonists act as very potent dipsogens in the watersaturated rat (cf. Falk and Tang 1974) and dog (Fitzsimons and Szczepanska-Sadowska 1974). The present study shows that this is not a general phenomenon among mammalian species. Infused intravenously in amounts previously shown to induce marked drinking in the dog, isoprenaline did not elicit drinking in the goat. The absence of dipsogenic response was apparently not due to a masking of thirst by sedative or nauseating effects of the drug, since the animals frequently consumed hay during the infusions. It can be concluded therefore, that a particular effect of β-adrenergic stimulation elicits an urge to drink in the rat and the dog, but not in the goat. As mentioned in the introduction, evidence has been produced that the activation of the renin-angiotensin system may be the ultimate cause of drinking occurring during β-adrenergic stimulation in the rat (Houpt and Epstein 1971). Like rats (Epstein, Fitzsimons and Rolls 1970), goats respond with drinking to the central application of angiotensin I₁ (cf. Andersson and Olsson 1973 and Fig. 3), and it appears likely that the renin-angiotensin system was stimulated to some extent during the isoprenaline infusions reported here. However, angiotensin I₁ obviously was not liberated in amounts sufficient to stimulate the thirst mechanism.

Isoprenaline induced drinking is apparently not due to an activation of the renin-angiotensin system in the dog (Fitzsimons and Szczepanska-Sadowska 1974). Furthermore, recent studies have shown that the administration of angiotensin I₁ blocking agents fails to attenuate β-adrenergically induced drinking in the rat (Tang and Falk 1974). It speaks against the idea that isoprenaline drinking is completely dependent upon the renin-angiotensin system in this species. Contribution of oropharyngeal factors of the kind responsible for normal prandial drinking in the rat cannot be excluded. The dipsogenic response to isoprenaline is attenuated in surgically desalivated rats (Falk and Bryant 1973). Furthermore, rats with lateral hypothalamic lesions continue to drink in response to isoprenaline (Lehr et al. 1967), although such lesions are supposed to abolish both "cellular" and "extracellular" (angiotensin-induced) thirst, leaving only the prandial drinking mechanism intact (Teitelbaum and Epstein 1962). Even the low dose of isoprenaline infused intravenously in the goat markedly reduced the flow of saliva (Fig. 2). In contrast to rats and dogs (Wolf 1958), goats are not prandial drinkers, and do not respond with drinking to atropine-induced reduction of the salivary flow. This seems to explain why the isoprenaline-evoked reduction in salivary flow did not induce the goats to take water. It also focuses the attention on the possibility that oro-
pharyngeal stimuli, related to diminished salivary secretion, might be the particular effect of β-adrenergic agonists which causes water intake in prandially drinking species.

Whether or not isoprenaline induced antidiuresis is mediated by ADH has been a question of some controversy. An antidiuretic response to the drug is obtained also in rats with congenital diabetes insipidus, where the contribution of ADH can be excluded (Levi, Grinblat and Kleeman 1971). A marked reduction in renal Na⁺ excretion takes place during isoprenaline antidiuresis in non-anesthetized, hydrated dogs, whereas ADH induced inhibition of the water diuresis is not accompanied by any decrease in the Na⁺ excretion (Klein et al. 1971). However, results obtained in anesthetized dogs (Schrier et al. 1972) suggest that the primary mechanism of the antidiuretic effect of β-adrenergic stimulation involves the integrity of the hypothalamo-neurohypophyseal system and the release of ADH.

In the hydrated goat the intravenous infusion of the low dose of isoprenaline did not reduce significantly the positive renal C₉H,O or the renal Na⁺ excretion. The hypotensive effect of these infusions was moderate and transient (Fig. 1, circles). However, infused at the rate of 0.3 μg/kg min⁻¹, isoprenaline caused a marked and sustained fall in arterial blood pressure which was accompanied by an inhibition of the water diuresis, characterized by negative renal C₉H,O and reduced renal Na⁺ excretion. (Fig. 1, dots). The cause of these renal effects appears to be two-fold. A rapid drop in the urine flow and the Na⁺ excretion was seen in spite of a rather slow development of negative renal C₉H,O. It indicates that reduced glomerular filtration rate contributed to the inhibition of the diuresis to some extent. Significant amounts of ADH were found in the urine during the periods of negative C₉H,O, which shows that release of ADH also was of major importance for the observed antidiuretic response to isoprenaline. Central β-adrenergic stimulation was apparently not the cause of this ADH-release since intraventricular infusions of isoprenaline did not elicit inhibition of the water diuresis. It has been shown that hypotensive drugs can induce antidiuresis by causing reflex release of ADH in response to the fall in arterial blood pressure (cf. Bisset and Lewis 1962). This appears to be the most likely explanation for the ADH-release observed during the intravenous infusions of the high dose of isoprenaline in the hydrated goat.

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References


Lishajko, F., To be published 1975.