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The difference in endocochlear and endolymphatic sac d.c. potentials in response to furosemide and canrenoate as diuretics

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Summary. We investigated the effects of furosemide, a loop diuretic, and canrenoate, an aldosterone antagonist, on the endocochlear potential (EP) and the endolymphatic sac potential (ESP) in the guinea pig. Furosemide produced no significant change in the ESP at a dose of 100 mg/kg after an intravenous infusion for 20 min. However, this dose decreased the EP to a negative level. Canrenoate produced no significant change in the EP at an intravenous dose of 300 mg/kg for 20 min, but it did decrease the ESP. The differences in the EP and ESP in the repsonse to the diuretics indicate a dissimilarity of the origin of both d.c. potentials in the endolymphatic space.

Key words: Furosemide – Aldosterone antagonist – Endocochlear potential – Endolymphatic sac

Introduction

It has been reported that an anoxia-sensitive positive d.c. potential of 15–20 mV is present in the intermediate portion of the endolymphatic sac (ES) [1, 8, 10]. Following anoxia, the ES d.c. potential (ESP) has been found to be different from the endocochlear potential (EP) and the ampullar endolymphatic potential of the semicircular canal (AEP). The EP and AEP have a negative potential during anoxia, whereas the ESP has no negative potential during anoxia [1, 8]. Ethacrynic acid is a loop diuretic not belonging to the furosemide family and is known to depress the EP and AEP to a negative value. This diuretic has also been found to produce little change in the ESP [8].

In order to achieve a better understanding of the difference in response of the EP and ESP to diuretics, we examined the effect of two different types of diuretics – furosemide, a loop diuretic, and canrenoate, an aldosterone antagonist – on the EP and ESP.

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Materials and methods

Animal preparation and recording technique. Adult albino guinea pigs (Hartley strain) with a positive Preyer's reflex, weighing between 230 and 420 g, were used. The animals were anesthetized with intraperitoneal sodium pentobarbital (33 mg/kg body weight), relaxed with suxamethonium chloride and artificially respirated through a tracheal cannula. Muscle relaxation was maintained with pancuronium bromide. Heart rates and rhythm were monitored by EKG in all animals throughout the experiment. The mean arterial blood pressure was recorded in selected animals from a catheter inserted into the right carotid artery and connected to a blood pressure transducer. The EP and ESP showed no difference between animals with and without an indwelling catheter in the carotid artery. A heating pad around the body maintained the rectal temperature at 36–38°C. Jugular vein cannulation was carried out for the administration of supplemental sodium pentobarbital and diuretic agents. The use of supplemental sodium pentobarbital and muscle relaxant (pancuronium bromide) produced no significant changes in the EP and ESP. Each animal's head was fixed with a head holder and the auditory bullae were opened to expose the round window membranes. The EP was recorded in the scala media through the round window membrane by means of a glass microelectrode. This electrode had a tip diameter of 1–2 μm and was filled with 160 m*M* KCl. The ES (mainly on the left side) was exposed extradurally by the posterior occipital approach [1, 8, 9]. The sigmoid sinus was detached medially from the medial surface of the temporal bone, allowing the ES to be identified just behind the sigmoid sinus. A glass microelectrode was inserted into the intermediate portion of the ES to measure the ESP. This latter electrode was filled with 154 m*M* NaCl instead of 160 m*M* KCl because it has been reported that the ES has an electrolyte composition similar to that of the extracellular fluid [8, 12]. A preliminary study of microelectrodes for measuring the ESP showed that a glass microelectrode filled with 154 m*M* NaCl gave similar ESP values and ESP responses to diuretics to an electrode filled with 160 m*M* KCl and 3M KCl. The microelectrode with a tip diameter of 2–6 μm was used in the present study, since it is more easily inserted into the ES through the tough walls surrounding the sac in the guinea pig. The connection of the microelectrode for the measurement of the EP and ESP was made via Ag/AgCl to an amplifier with a high-input impedance of 10^{15} Ω (WPI FD 223). The reference electrode was an Ag/AgCl electrode placed on the neck muscles. The EP and ESP were concurrently recorded in three animals following the administration of each diuretic. All experiments were carried out in an electrically shielded booth.

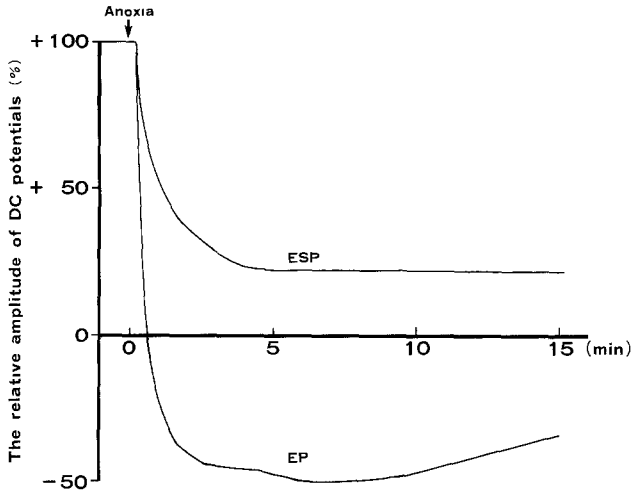

Fig. 1. Response of the endocochlear potential *(EP)* and endolymphatic sac potential *(ESP)* to anoxia in a concurrent recording. The change in the potentials is expressed as a percentage of the preanoxic value (+76 mV for the EP and +17.5 mV for the ESP)

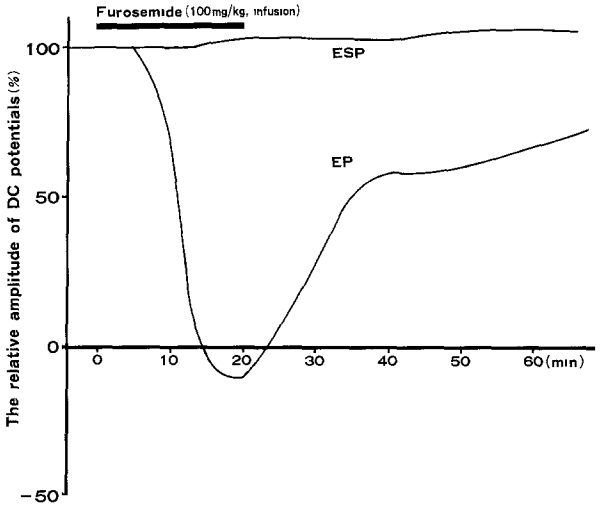

Fig. 2. Effect of furosemide (100 mg/kg) on the EP and ESP after a 20-min infusion. The change in the potentials is expressed as a percentage of the pre-infusion value (+95 mV for the EP and +16 mV for the ESP)

Drug administration. Furosemide (100 mg/kg, Hoechst, Frankfurt, FRG) and potassium canrenoate (300 mg/kg, Searle, Chicago, Ill., USA) were dissolved in 4 ml saline and infused over a 20-min period using an infusion pump (Terumo STC-521, Tokyo, Japan) through a catheter inserted into the jugular vein of the animal being tested.

Data analysis. Values are presented as means ± SE. Student's *t*-test was used to determine the statistical difference between paired groups.

Results

The EP and ESP were +83.6 ± 1.9 mV (n = 10) and +19.0 ± 1,6 mV (n = 17), respectively. The amplitude of the ESP was comparable to those previously reported in the pigmented guinea pig [1, 8, 10].

Table 1. Comparison of the effect of furosemide (100 mg/kg) on the endocochlear potential (EP) and endolymphatic sac potential (ESP) after a 20-min infusion (n = 4)

	EP (mV)	ESP (mV)
Pre-infusion	+84.5 ± 3.7	+17.3 ± 3.7
Post-infusion	−10.8 ± 6.6*	+17.3 ± 4.4

* Significant different from the pre-infusion value ($P < 0.01$)

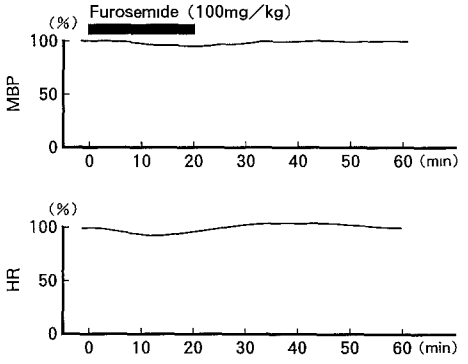

Fig. 3. Influence of furosemide infusion (100 mg/kg) on the mean arterial blood pressure *(MBP)* and heart rate *(HR)* after a 20-min infusion

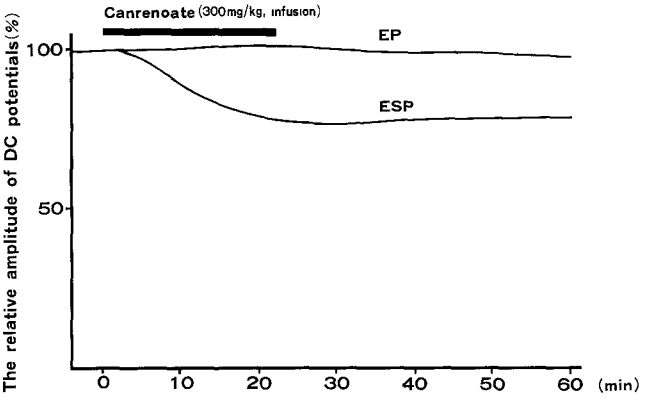

Fig. 4. Effect of canrenoate (300 mg/kg) on the EP and ESP after a 20-min infusion. The change in the potentials is expressed as a percentage of the pre-infusion value (+93 mV for the EP and +30.5 mV for the ESP)

A typical response of the EP and ESP to anoxia is shown in Fig. 1. Anoxia was caused by stopping the respirator. The EP decreased to a negative value after inducing anoxia, whereas the ESP was depressed but not to a negative value. The absence of a negative potential in the ESP is in accordance with previous reports [1, 8, 10].

Figure 2 shows concurrent recordings of the EP and ESP in the 20-min infusion of furosemide (100 mg/kg). After the start of the infusion, the EP gradually declined to a negative value at approximately 15 min. After the infusion had been stopped, the EP gradually recovered. Furosemide produced no change in the ESP (Table 1). The time taken to reach the minimum level of the EP after the infusion was 18.8 ± 1.3 min (n = 4). During this

Table 2. Comparison of the effect of canrenoate (300 mg/kg) on the EP and ESP 5 min after a 20-min infusion

	EP (mV)	ESP (mV)
Pre-infusion	$+83.0 \pm 2.2$ ($n = 6$)	$+20.6 \pm 2.3$ ($n = 10$)
Post-infusion	$+81.8 \pm 2.6$ ($n = 6$)	$+15.6 \pm 1.7$ ($n = 10$)*

* Significantly different from the pre-infusion value ($P < 0.001$)

period, a temporary, mild decrease occurred in the mean arterial blood pressure and heart rate (Fig. 3).

Figure 4 shows concurrent recordings of the EP and ESP during a 20-min infusion of canrenoate (300 mg/kg). Canrenoate induced no change in the EP, whereas it decreased the ESP (Table 2). After the start of the infusion, the ESP gradually decreased and reached a stable value at 25 min. After the infusion had been stopped, the ESP failed to recover. The time taken to reach the minimum value of the ESP after starting the infusion of canrenoate was 21.2 ± 1.8 min ($n = 10$). The infusion produced little change in the mean arterial blood pressure and heart rate in most animals.

Discussion

Furosemide induced no significant change in the ESP at a dose which produces a decline in the EP to a negative value. It has been reported that ethacrynic acid at a dose of 100 mg/kg induces no change in the ESP [8], indicating that loop diuretics show no direct effect on the ESP. Erwall et al. [4] found that the cellular changes induced in the ES by loop diuretics may not be a result of a primary toxic effect per se, but may be secondary to alterations in fluid and ion homeostasis in the rest of the inner ear. These findings are in agreement with our present results.

Like spironolactone, potassium canrenoate is a steroid derivative which is an indirect aldosterone antagonist. Spironolactone and potassium canrenoate are both metabolized to canrenone, which is an active aldosterone antagonist. Potassium canrenoate can be regarded as the potassium salt of one spironolactone metabolite [2]. Unlike spironolactone, potassium canrenoate is a water-soluble substance that can be administered parenterally. In the present study, canrenoate produced no change in the EP at a dose which produced a decrease in the ESP. That no significant changes occurred in the EP or the circulatory systems of our animals shows that the changes induced by canrenoate in the ESP are specific. Our results suggest that aldosterone may play an important role in the generation and modulation of the ESP but not in that of the EP. This hormone has been reported to stimulate sodium absorption in certain mammalian epithelia, such as the cortical collecting tubule [7, 11] and the colon [3, 5]. Failure of the EP to be changed by the aldosterone antagonist canrenoate supports the notion that sodium ion transport plays no important role in the generation and maintenance of the EP [6, 13]. Our previous report favors the possibility that sodium ions may be actively pumped out of the ES, and that this transport may be involved in the origin of the ESP [8]. In this system, sodium ion transport in the ES may play an important role in the longitudinal flow of endolymph in the inner ear. It is possible that aldosterone may stimulate the sodium ion transport in the ES, resulting in the regulation of the longitudinal flow of the endolymph.

Our present study has demonstrated the difference in the EP and ESP response to different kinds of diuretics, suggesting the dissimilarity of the character of both d.c. potentials in the endolymphatic space.

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