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The rabbit as an intracavernous injection study model

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Abstract We investigated the feasibility of using the rabbit as an animal model for intracavernous injection studies. The rabbit, having a penile structure rather similar to that of humans, offers the advantage of being a strain-specific, adequately sized, and easily controlled experimental animal. Using intracavernous injections of the two vasoactive drugs prostaglandin E_1 (PGE_1, 0.2–1.6 μg/kg) and papaverine (PAP, 0.25–1 mg/kg), which have been commonly used in the management of erectile dysfunction in man, increases intracavernous pressure (ΔICP) were induced. After intracavernous injection of PGE_1, the maximal ΔICP ranged from 18 to 44 mmHg (mean 29.25 ± 7.85 mmHg) with a duration of tumescence from 3.1 to 13.3 min (mean 8.61 ± 3.71 min). Intracavernous injection of PAP also induced increases in ICP, with a maximal ΔICP ranging from 24 to 56 mmHg (mean 43.5 ± 11.35 mmHg) and a duration of tumescence from 5.3 to 15 min (mean 10.25 ± 3.39 min). The systemic blood pressures were unchanged after all intracavernous injections. In addition, administration of cAMP antagonist in combination with PGE_1 inhibited the relaxing effects of PGE_1 in a dose-dependent manner. Our results suggest that the effects of vasoactive drugs on the rabbit's corpus cavernosum are similar to those in humans; thus the rabbit model is a suitable alternative for further physiological and pharmacological studies of penile erection.

Key words Rabbit · Erection · Prostaglandin · Papaverine

Introduction

In recent years, the neurovascular mechanisms of penile erection have been widely studied. Many researchers have defined several possible neurotransmitters which may be involved in this relaxing event. The role of the sinusoidal endothelium in the relaxation of the cavernosal smooth musculature has also been emphasized. In a clinical setting, recent progress in the treatment and diagnosis of erectile dysfunction has been based on the pharmacological effect of the intracavernous injection of vasoactive drugs. Prostaglandin E_1 (PGE_1) and papaverine (PAP) have been accepted as the most common drugs used for intracavernous injections. PGE_1 induces the accumulation of intracellular cyclic adenosine monophosphate (cyclic AMP), then causing smooth muscle relaxation [1, 8, 16]. The action of PAP is to relax smooth muscle directly, which is achieved through reducing intracellular calcium concentration and cyclic nucleotide phosphodiesterase activity [2]. However, the acceptance rate of intracavernous injection therapy at our institute is 24.7% [11]. The reasons for patient drop-out include the idea of injection itself, an unsatisfactory response, pain and financial considerations. Thus, the treatment of erectile dysfunction still requires improvement. Further investigation into the neurophysiology and pharmacology of penile erection is continuing.

For studies of penile erection, we believe that an in vivo animal model is superior to an in vitro study in an organ bath chamber. Advantages include the ability to perform continuous monitoring of the intracavernous pressure (ICP), duration of erection, and systemic arterial pressure, fewer possible side effects and better reproducibility. Many experimental animals have been employed in in vivo studies of penile erection, such as dogs, monkeys, cats, rats and rabbits [3–5, 12–14]. However, the important differences between species should be considered for every specific study, and in addition there are many obstacles to satisfactory experimental performance. Economic and ethical considerations have led to a decrease in the use of the monkey as an experimental material. The dog, which has two separate corpora, a bone at the proximal part of the

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penis and venous drainage via the cavernous veins only, is rather different anatomically from humans [3, 6]. The cat has less strain-specific differences than the rat or rabbit. Although the rat has the advantage of being less expensive, strain-specific and having similar behavior and endocrine characteristics of penile erection to humans [4, 9], the small size of the cavernous bodies result in technical difficulties in intracavernous injection and ICP recordings. The rabbit is similar to the rat as regards cost and availability. Stief et al. [15] reported that the rabbit was a suitable experimental animal for the neurophysiological studies of penile erection in vivo. However, in their study, the lack of intracavernous injection data limited the use of that rabbit model to the local effects of pharmacological agents. Although it has been suggested that in the rabbit PGE_1 is less effective in inducing penile erection, which may be due to the lack of a PGE_1 receptor [14], the aim of this study was to evaluate the feasibility of using the rabbit as a possible animal model for intracavernous injection studies with the vasoactive drugs PGE_1, and PAP, which are commonly used in impotent men.

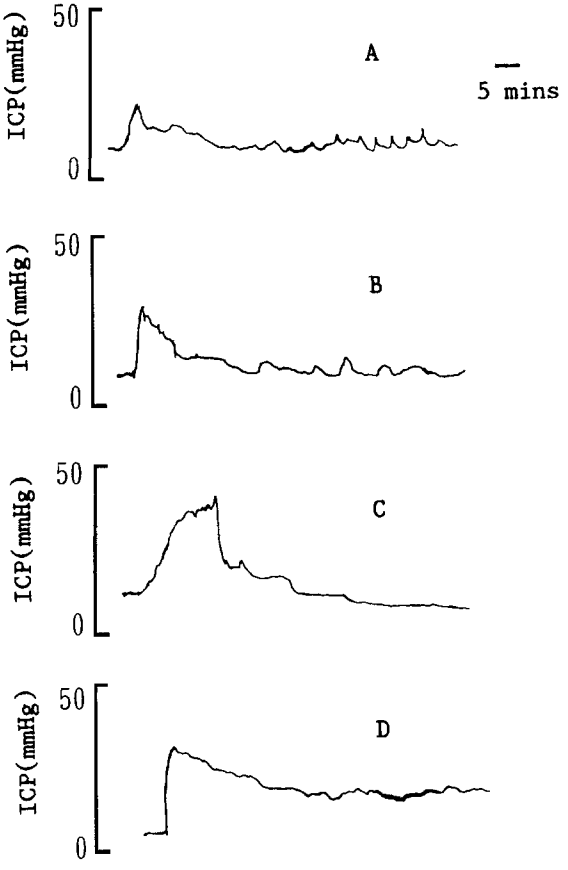

Fig. 1A–D Representative time-course change after PGE_1 injections in different doses (**A** 0.5 μg, **B** 1 μg, **C** 2 μg, **D** 4 μg). Intracavernous injection of 2 μg PGE_1 led to a peak ICP of 42 mmHg

Materials and methods

Animals

A total of 18 male New Zealand white rabbits weighing 2–3 kg were used for the investigation. After sedation with an intramuscular injection of ketamine 10 mg, the rabbits were anesthetized with intraperitoneal pentobarbital sodium 30 mg/kg. Anesthesia was maintained at 10 mg/kg as needed. The animals breathed spontaneously. The rabbits were then placed in the supine position, and the body temperature was maintained at 37 °C using a heating pad and lamp. The femoral artery on one side was cannulated for monitoring of continuous systemic arterial pressure (SAP), mean systemic arterial pressure (MSAP) and heart rate (HR) via a Gould 23 ID pressure transducer. Under sterile conditions, the skin overlying the penis was incised and the corpora cavernosa was exposed at the root of the penis. A 25-gauge needle was inserted into the corpus cavernosum for pressure recording (Grass Polygraphy RS3600). The needle was connected to a three-way stopcock, thus permitting the intracavernous injection of drugs. The tube was filled with heparinized saline (50 IU/2–3 h) to prevent clotting.

Chemicals and drugs

We used the following drugs: papaverine hydrochloride (PAP, U-Liang Pharmaceutical, Taiwan); prostaglandin E_1 (PGE_1, Ono Pharmaceutical, Japan); cyclic AMP-s Rp isomer (Boehringer Mannheim, Biochemica, Germany) and normal saline (YF Chemical, Taiwan).

Intracavernous injection of vasoactive drugs and normal saline

In eight rabbits, increasing concentrations of PAP (0.25–1 mg/kg) and PGE_1 (0.2–1.6 μg/kg) were injected intracavernously in a volume of less than 0.15 ml. Normal saline in increasing volumes (0.02–0.2 ml) was injected in four rabbits as a control group. In a pilot study at our institute, the volume of both corpora, which are connected, measured 0.6 ± 0.17 ml ($n = 12$). The effects of vasoactive drugs and normal saline were then determined from the rise in

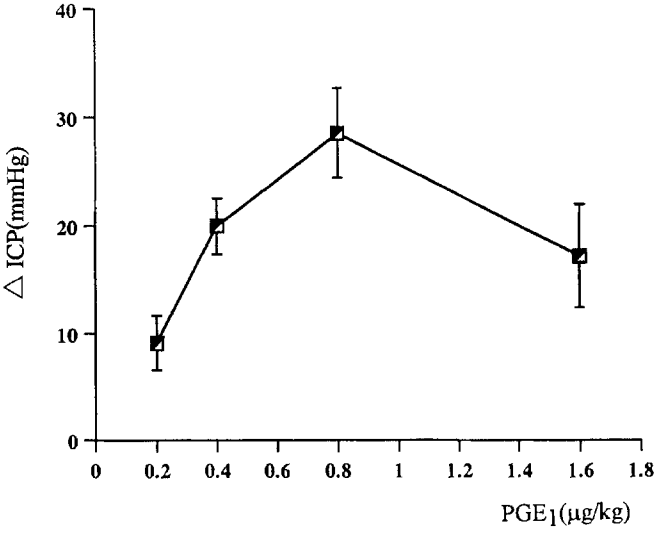

Fig. 2 Administration of PGE_1 in increasing doses induced a dose-dependent elevation in ΔICP; however, subsequent administrations of a higher dose (1.6 μg/kg) resulted in diminished changes ($n = 8$)

Table 1 Peak increased intracavernous pressure (ΔICP) and duration of tumescence response to PGE_1 and PAP ($n = 8$)

Rabbit	ΔICP (mmHg)		Duration (min)	
	PGE_1	PAP	PGE_1	PAP
1	44	56	13.3	15
2	18	42	5.6	14
3	32	24	11.7	5.3
4	24	56	3.1	9
5	30	40	12.5	8.4
6	28	32	9.6	9.2
7	24	48	6	13.1
8	34	50	7.1	8
Mean ± SD	29.25 ± 7.85	43.50 ± 11.35	8.61 ± 3.71	10.25 ± 3.39

P (PGE_1 vs PAP, paired Student's *t*-test): ΔICP 0.016, duration 0.41

ICP (ΔICP) and duration of tumescence. The doses showing the best response were used to compute the results. In order to avoid the effect of the previous drug, the cavernous body was flushed with 0.2 ml normal saline before each injection and the time interval between each injection was at least 1 h.

The effect of cAMP-s, Rp isomer in combination with PGE_1

It is believed that PGE_1 stimulates the formation of cyclic AMP, then induces the relaxation of cavernosal smooth muscle. Cyclic AMP-s Rp isomer is a membrane-penetrating cAMP antagonist that acts as a competitive inhibitor of cAMP-dependent protein kinase types I and II. In six rabbits, following the intracavernous injection of PGE_1 0.8 μg/kg as a baseline control, cAMP-s, Rp isomer in increasing doses (0.02–0.08 μmol/kg) was injected intracavernously in combination with PGE_1. The changes in ICP were then evaluated.

Statistics

Data were expressed as mean ± SD and were analysed for statistical significance by Student's *t*-test. *P* values less than 0.05 were considered significant.

Results

Baseline value

In the pilot study, the best site for intracavernous injection was on the mid portion of the penis, dorsolaterally, just proximal to the attachment of the foreskin. Three rabbits were killed after ICP recording, and the cavernous bodies were meticulously dissected to confirm the proper placement of the needle. We ascertained that the best depth for needle insertion was 5–6 mm. The baseline ICP recorded in 22 rabbits was 13.1 ± 4.1 mmHg. Although transient rises or an unstable ICP following needle insertion were found occasionally, the ICP restabilized within 10–20 min.

Effects of vasoactive drugs and normal saline

After intracavernous injections of PGE_1 in eight rabbits, the maximal ΔICP ranged from 18 to 44 mmHg (mean 29.25 ± 7.85 mmHg) with a duration of

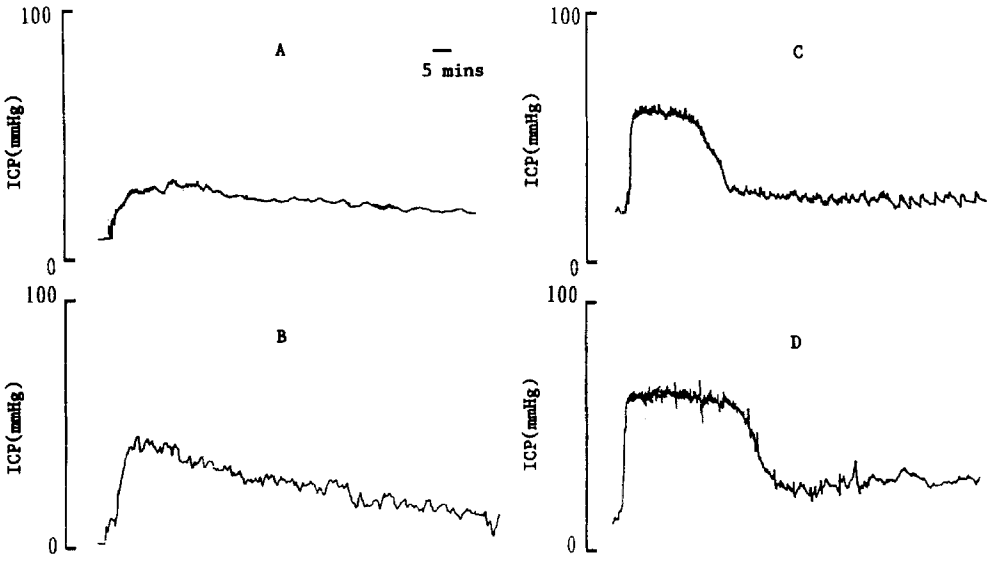

Fig. 3A–D Representative time-course change after PAP injections in different doses (**A** 0.75 mg, **B** 1.5 mg, **C** 2.25 mg, **D** 3 mg). Intracavernous injection of 3 mg PAP led to a peak ICP of 67 mmHg

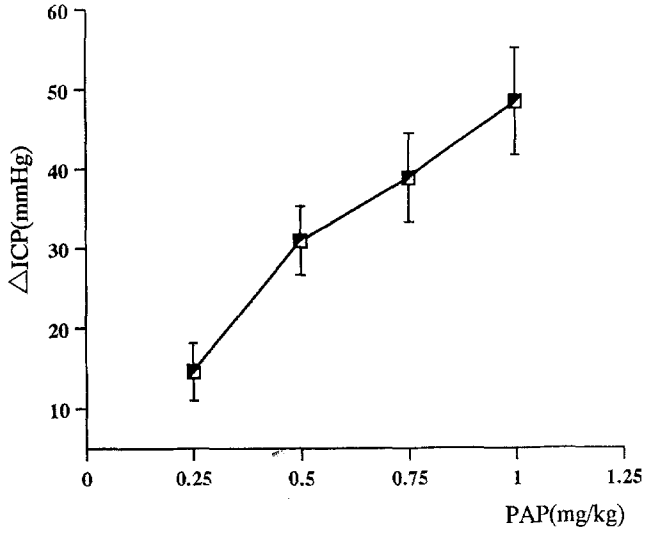

Fig. 4 Administration of PAP in increasing doses induced a dose-dependent elevation in ΔICP ($n = 8$)

Fig. 5 Representative ICP changes after intracavernous injections of normal saline in different doses (from 0.05 to 0.20 ml)

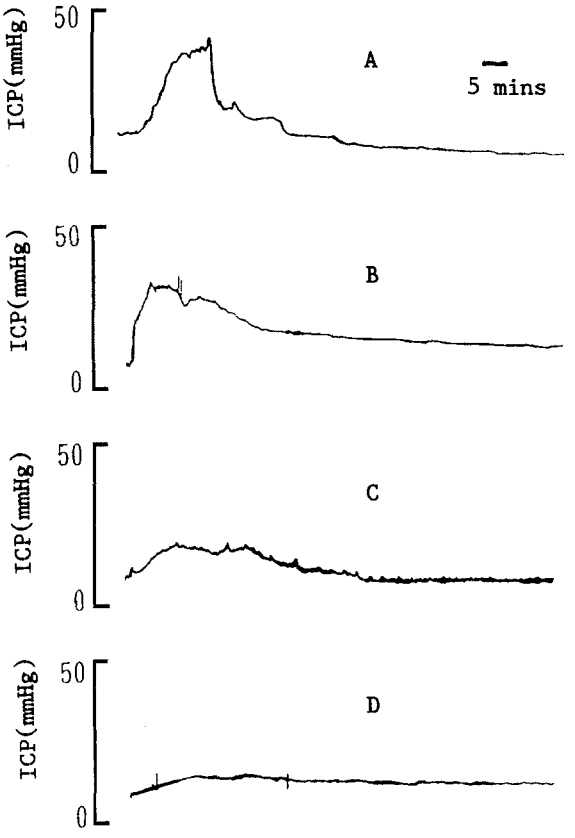

Fig. 6A–D Representative time-course changes after intracavernous injections of PGE_1 alone, **A**; and in combination with cAMP-s, Rp isomer in increasing doses (**B** 0.04 μmol, **C** 0.08 μmol, **D** 0.16 μmol)

tumescence from 3.1 to 13.3 min (mean 8.61 ± 3.71) (Table 1). Figure 1 shows the time course changes in ICP after intracavernous injections of PGE_1. During the injection periods, the SAP, MSAP and HR were unchanged. We also found in some cases tumescence of the penile shaft (straightening), but not full erection. Administration of PGE_1 in increasing doses (0.2–0.8 μg) induced a dose-dependent elevation in ICP; however, subsequent injection of a higher dose (1.6 μg/kg or more) resulted in a decrease in ICP or no response (Fig. 2). Intracavernous injection of PAP also induced rises in ICP in eight rabbits, with a maximal ΔICP ranging from 24 to 56 mmHg (mean 43.5 ± 11.35 mmHg) and duration of tumescence from 5.3 to 15 min (mean 10.25 ± 3.39 min) (Table 1). Figure 3 shows the time-course changes in ICP after intracavernous injections of PAP. SAP, MSAP and HR were also unchanged. Administration of PAP in increasing doses (0.25–1 mg/kg) resulted in a dose-dependent elevation in ICP, with 1 mg/kg giving the best response (Fig. 4). The effects of PAP seemed to be more potent than those of PGE_1 on ΔICP but not on duration of tumescence ($P = 0.016$, 0.41, respectively) (Table 1). Intracavernous injection of normal saline induced a transient rise in ICP in a dose-dependent manner (Fig. 5). Nevertheless, the pressure rises often returned to the resting level within 1 min and the spike-like pressure tracing curves were different from those of PGE_1 and PAP. We believe that the transient rise in ICP was due to the volume effect of injected normal saline.

Effects of cAMP-s, Rp isomer in combination with PGE_1

After intracavernous injection of PGE_1 0.8 μg/kg as a baseline control, cAMP-s, Rp isomer was injected in increasing doses (0.02–0.08 μmol/kg) in combination with PGE_1 (0.8 μg/kg). Figures 6 and 7 illustrate that administration of cAMP-s, Rp isomer inhibits the effect of PGE_1 in a dose-dependent manner.

Discussion

The purpose of an animal model is to provide information which is applicable to a better understanding and

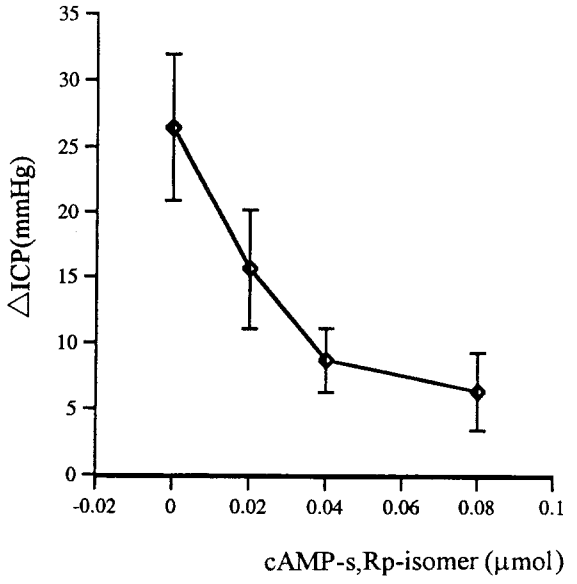

Fig. 7 Administration of PGE_1 (0.8 μg/kg) and in combination with cAMP-s, Rp isomer in different doses (from 0 to 0.08 μmol/kg) results in decreased changes in ICP ($n = 6$)

further studies of human disease. Leader et al. [10] suggested that a good experimental animal model should be accurately reproducible, exportable, easily handled, large enough for multiple biopsies, available to multiple investigators, survive long enough to be usable and fit into the available animal facilities of most laboratories. Undoubtedly, the present rabbit model fits all of the above criteria. In our studies, the feasibility of injecting vasoactive drugs intracavernously was similar to that in humans. In combination with the rabbit model for neurourological studies as used by Stief et al. [15], we believe that the rabbit model can be a suitable alternative for further physiological, pathophysiological and pharmacological studies of penile erection.

In the present study, intracavernous injection of PGE_1 and PAP resulted in rises in ICP with a maximal ΔICP of 29.25 ± 7.85 and 43.5 ± 11.35 mmHg, respectively. Neither PGE_1 nor PAP induced full erection in this in vivo model, the peak ICP being only 25% and 36% of MSAP, respectively. Obviously, the relaxing effects of PGE_1 and PAP in the rabbit's corpus cavernosum are less than those in the human. We hypothesize that the different effect between rabbits and humans may be due to several factors. First, the species difference may result in different biochemical behavior of cavernosal tissue; thus the sensitivity to vasoactive drugs must differ. Second, PGE_1 in the human may act mainly on the prostaglandin E subtype EP_2 (relaxation) receptor. In the rabbit, PGE_1 may act at other receptor sites such as EP_1 (contraction) and EP_3 (contraction). The actions of EP_1 and EP_3 may then nullify the EP_2 relaxant effect. On the other hand, the lack of PGE_1 receptor in the rabbit's erectile tissue is also possible. Third, the main intracellular pathway of penile erection in the rabbit is not the cAMP system, and so the relaxing effect of PGE_1 is limited. Nevertheless, all of these factors remain to be elucidated.

From a review of the literature, only Stackle et al. [14] have studied the erectile response of the intracavernous injection of vasoactive drugs in the rabbit. Their report showed that PAP was more effective than PGE_1 in inducing penile erection in the rabbit, as also shown in our studies. Although some of the results we obtained were similar to those of their experiments, some notable differences exist. In their studies, 5 out of 25 PAP injections gave full penile erection, and PGE_1 induced partial erection (25% of full erection) in only 7 of 25 injections. In contrast with our findings, neither PAP nor PGE_1 injection induced full erection, and the relaxing effect of PGE_1 seemed to be much prominent than theirs. It must be mentioned that in their studies the erectile response was expressed using a subjective visible grading scale (i.e. percentage of full erection), but not ICP. It is believed that the change in erectile response could not be exactly detected by that means. Therefore, further studies are necessary to corroborate these data.

Since the diameter of the unilateral cavernous body of the rabbit (2.5–3.5 mm) is much larger than that of a 25-gauge needle (0.5 mm), the needle insertion itself can be achieved easily and accurately. However, a more difficult problem is posed by the maintenance of the needle in a satisfactory position and the patency of the tubing during penile erection. In our experience, certain points should be kept in mind to avoid possible pitfalls in ICP recordings. First, the rabbit must be studied under at least light surgical anesthesia to prevent the animal from moving. Monitoring the depth of anesthesia should be done by checking the pedal withdraw reflex every 2 h [7]. Second, heparin (50 IU) should be given through the tubing system every 2–3 h to prevent clotting. The straight, flat tracing curve at any level always indicates obstruction. Third, the blood must be aspirated from the catheter without difficulty. Fourth, the cavernous body is flushed with 0.2 ml normal saline before each injection to ascertain the needle position. If a visible swelling occurs in adjacent tissue, the position of the needle should be checked.

There are many examples where the elucidation of pathophysiology via the animal model is ahead of our understanding of the human disease. Although male sexual dysfunction has been extensively investigated using some animal models, its treatment is still far from ideal. The present rabbit model, we believe, can be used to elucidate agents of potential use in the treatment of erectile dysfunction.

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