ORIGINAL RESEARCH—BASIC SCIENCE

Effects of Adenosine Monophosphate Used in Combination with L-Arginine on Female Rabbit Corpus Cavernosum Tissue

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ABSTRACT

Introduction. Sexual dysfunction is significantly more prevalent in women than in men. However, to date, no satisfactory oral treatment is yet available.

Aim. The aim of this study was to study the effects of adenosine monophosphate (AMP) alone or its combination with L-Arginine on the relaxation of the female rabbit corpus cavernosum.

Methods. Cylinder strips from the corporal body of the excised clitoris from female New Zealand White rabbits were incubated in Krebs solution. Phenylephrine (PE) precontraction was achieved, then the drugs AMP and L-Arginine were administered either independently or in sequential combinations to the strips under precontracted conditions.

Main Outcome Measures. Contraction percentages were compared.

Results. When precontraction was induced by PE 8 μM or 20 μM, AMP was shown to induce relaxation up to 25% in a dose-dependent manner. The relaxation induced by L-Arginine reached 15.6% at 5.10^{-4} M vs. 16.5% at AMP 5.10^{-4} M under the same experimental conditions. Nitric oxide (NO) synthase inhibitor N-nitro-L-arginine strongly inhibited the relaxing effect provoked by AMP, suggesting that the action mechanism of this nucleotide is related to the NO pathway. The combination of L-Arginine at 5.10^{-4} M with AMP at different doses ranging from 5.10^{-4} M to 10^{-3} M significantly amplified the relaxing response up to 40.7% and 58%, respectively.

Conclusions. Our results demonstrate that AMP induces a relaxing effect on the female rabbit corpora. They also show that L-Arginine and AMP can potentiate each other and that a synergistic effect can be obtained by their combined use. Because only slight differences exist between both sexes in response to NO donors and/or nucleotide purines or in their use together, it is very likely that close biochemical mechanisms, although not to the same degree and not quite similar, are involved in the engorgement of the penis and the clitoris of New Zealand White rabbits. **Stücker O, Pons C, Neuzillet Y, Laemmel E, and Lebret T. Original research-sexual medicine: Effects of adenosine monophosphate used in combination with L-Arginine on female rabbit corpus cavernosum tissue. Sex Med 2014;2:1–7.**

Key Words. Purines; AMP; ATP; L-Arginine; Female Corpus Cavernosum Relaxation; Nitric Oxide

Introduction

Over the past two decades, greater attention has been paid to male impotence than sexual disorders in women. Scientific investigation has primarily focused on male sexual dysfunction. In comparison, however, there has been a dearth of research concerning the physiology and the functional mechanisms underlying female sexual dysfunction (FSD). In many respects, the epide-

miological study published in 1999 by the National Health and Social Survey [1] was the first major study to address this problem. It confirmed that women not only suffer from sexual dysfunction, but it also revealed that the prevalence of this disorder is even more significant in women than in men (43% vs. 31%, respectively). Since then, FSD has been identified as being of widespread public health concern driven by many possible underlying causes responsible for desire, arousal, orgasm, or pain disorders and in some cases, a combination of these conditions. It is now accepted that these disorders deserve a specific therapeutic approach that should not be limited to psychosocial and relationship aspects alone but sometimes extended to the use of specific drugs as in the case of female sexual arousal disorders (FSAD) and hypoactive sexual desire. This has given rise to several scientific projects to assess the problem of FSD. However, to date, no reported study, to our knowledge, has emerged as a satisfactory therapeutic solution. Further studies are required to broaden our knowledge in the field of FSD. Therefore, we were prompted to conduct a study based on a program dedicated to the search of vasoactive drugs, which would exclusively focus on the treatment of FSAD.

In a previously reported study, we evaluated the effect of adenosine monophosphate (AMP) combined with L-Arginine on the mechanism of erection in the male rabbit penis [2]. We were able to show a synergistic effect because of these combinations on strips of smooth muscle corpus cavernosum (CC). The present study was designed to evaluate the effect of the same compounds alone, or in combination, on the female New Zealand rabbit clitoral CC. The existence of close similarities between the rabbit and the male human erectile tissues, which are greater than that between men and other mammal erectile specimens, has been widely documented [3–6]. Morphological and physiological similarities between male and female New Zealand rabbit CC have also been described [7,8]. Therefore, the female New Zealand White rabbit was selected, for this preliminary approach, as it was considered the most suitable animal model.

Materials and Methods

Strip Preparation

Healthy female New Zealand White rabbits weighing 2–2.4 kg and 10–12 weeks of age were sacrificed by an overdose of sodium pentobarbital injected into the marginal vein of the ear. The clitoris was excised from surrounding external genitalia and placed in Krebs solution equilibrated with 95% O_2 and 5% CO_2 at 4°C. The corporal body was dissected free from the tunica and then sliced into four cylinder strips (two proximal, two distal) of approximately 4–5 mm each as previously described by other authors [9].

Tissue Bath Setup

Longitudinal strips were attached to force transducers using silk sutures. They were incubated in Krebs solution in 25 mL organ baths, equilibrated with 95% O_2 and 5% CO_2 and maintained at 37.5°C. Isometric strip tension was recorded through an acquisition unit (MP30, BioPac, Goleta, CA, USA) connected to a computer database. After mounting, each strip was allowed to equilibrate with a basal tension between 0.20 g and 0.25 g for at least 2 to 3 hours. During this time, the harvested segments were washed regularly.

Drug Administration

Phenylephrine (PE) (Sigma, Saint-Louis, MO, USA) precontraction was achieved by the addition of 8 or 20 µM (as indicated by the manufacturer) to each bath in order to reach a stable strip contracting effect. Although 8 µM PE was generally considered sufficient with no detectable change at 20 µM PE, we preferred to use the 20 µM dosage in order to obtain the highest possible precontraction effect. Drugs AMP (Interchemica S.r.l., Strambino, Italy) and L-Arginine (Sigma-Aldrich, Saint-Quentin-Fallavier, France) were administered either independently or in sequential combinations (AMP at different doses, then L-Arginine at different doses, then the combination AMP–L-Arginine) to the strips under precontracted conditions. The final bath concentrations were between 10^{-4} M and 10^{-3} M. Nitric oxide (NO) synthase inhibitor N-nitro-L-arginine (L-NNA) at the dose of 5.10^{-4} M was also administered to the strips before treatment with AMP.

Statistical Analysis

Contraction percentages were compared between groups by a variance analysis in repeated measurements. A value of $P < 0.05$ was considered significant in all tests. All results are expressed as mean ± standard error of the mean.

Results

The maximal relaxing effect after addition of active substances was measured at each stage (for
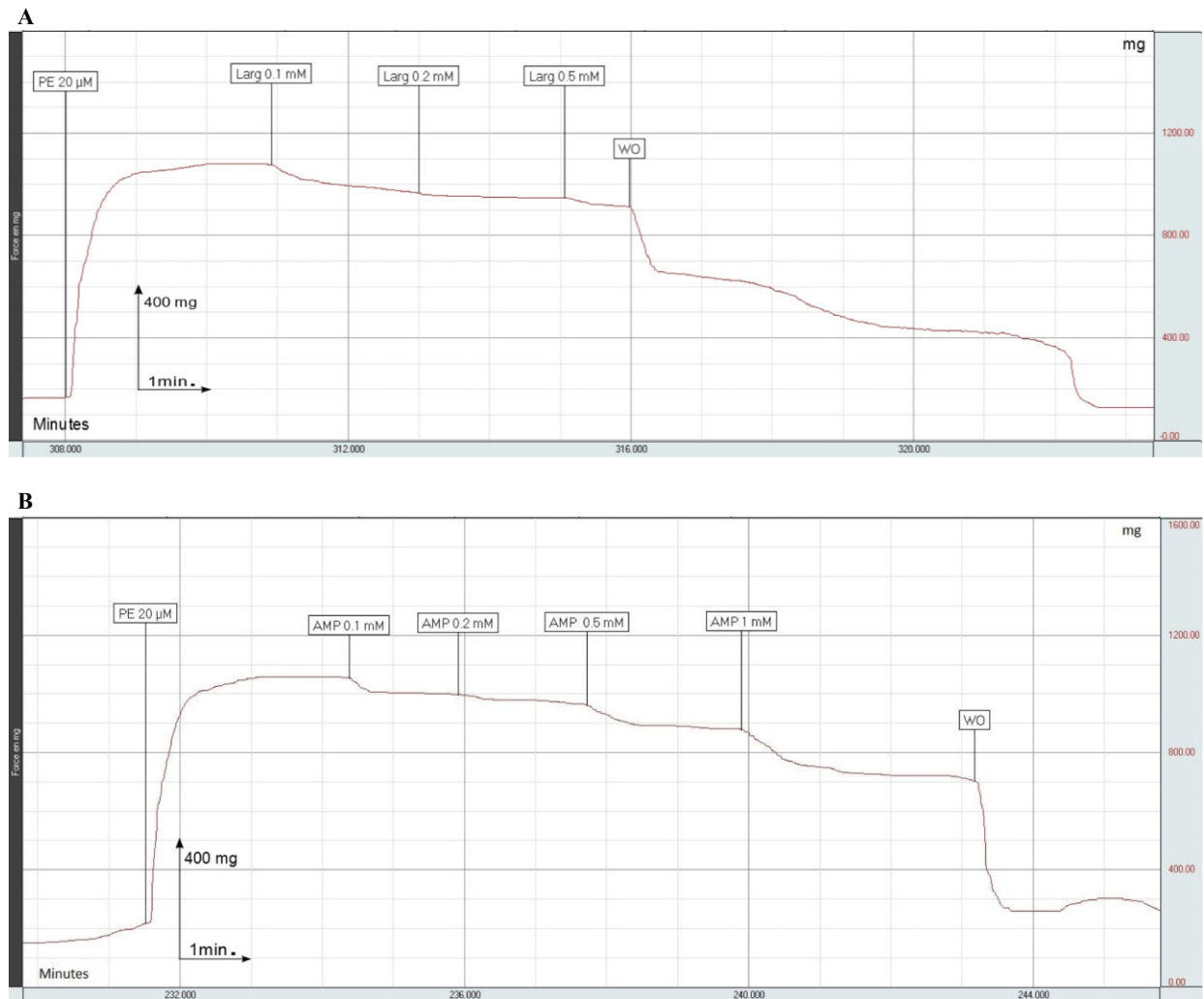
Figure 1 Dose-response curve over time for L-Arginine (A) and adenosine monophosphate (B). AMP = adenosine monophosphate; Larg = L-Arginine; PE = phenylephrine; WO = wash-out

each concentration) and was reached in approximately 1 minute as shown in Figure 1. The number of single strips for each experiment was n = 12.

AMP-Induced Relaxation

Muscles were precontracted by PE 20 µM. Exposure of this preparation to AMP led to a significant ($P < 0.001$) relaxation level that reached 11.7% at 5.10^{-4} M and 25% at 10^{-3} M (see Figure 2).

When L-NNA was used before AMP, the relaxation produced by AMP at 5.10^{-4} M and at 10^{-3} M was strongly inhibited, i.e., limited to 6.7% and 6.9%, respectively. The relaxing effect was significantly different with and without L-NNA, especially at AMP 10^{-3} M ($P = 0.009$).
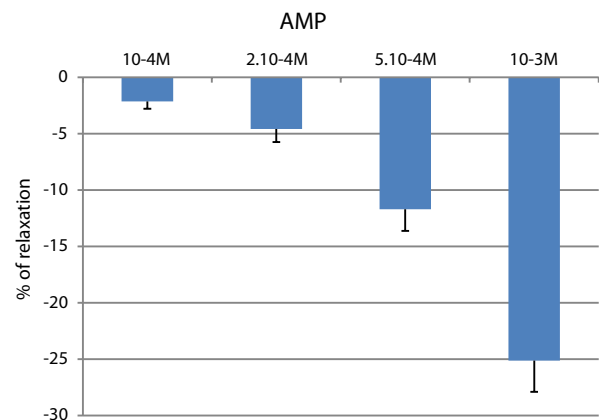

Figure 2 AMP-induced relaxation on corpus cavernosum precontracted with phenylephrine (20 µM). Vertical bars indicate SEM, (n = 12). AMP = adenosine monophosphate; SEM = standard error of the mean
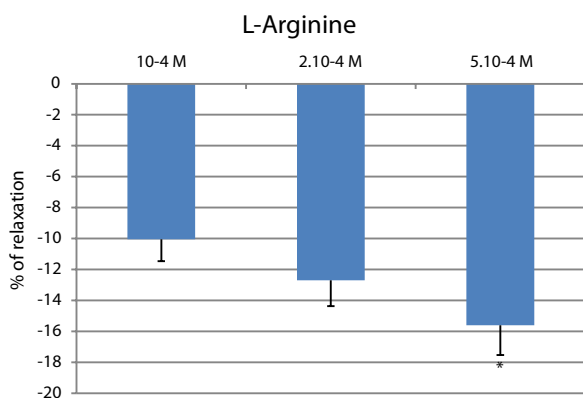
L-Arginine

Figure 3 L-Arginine-induced relaxation on corpus cavernosum precontracted with phenylephrine. Vertical bars indicate SEM, (n = 12). SEM = standard error of the mean

L-Arginine-Induced Relaxation

When L-Arginine was used to induce relaxation after a precontraction to PE, the relaxation was 15.6% at 5.10^{-4} M in a concentration-dependent way ($P < 0.001$). The results are presented in Figure 3.

AMP/L-Arginine-Induced Relaxation

Precontraction with PE was induced before a concentration-dependent curve to AMP (5.10^{-4} and 10^{-3} M) followed by different L-Arginine concentrations of (10^{-4}, 2.10^{-4}, and 5.10^{-4} M). The results (see Figure 4) show that under these experimental conditions, AMP induced a significant ($P < 0.0001$) relaxation (16.5% at 5.10^{-4} M and 37% at 10^{-3} M). When L-Arginine 5.10^{-4} M was added, the AMP-induced relaxation was significantly enhanced; at AMP 5.10^{-4} M, a 41% relaxation was reached (vs. 16.5% without L-Arginine) and at 10^{-3} M, a 58% relaxation was reached (vs. 37% without L-Arginine).

A *synergistic effect* was demonstrated with these two compounds as the final result was superior to the addition of the two effects obtained separately (15.6% for L-Arginine alone). To confirm this result, the response induced by L-Arginine alone was substracted from that induced by L-Arginine with AMP and the result compared with AMP without L-Arginine. Using paired tests and comparing *AMP without L-Arginine* to *AMP with L-Arginine*, a significant difference was found between the two, whatever the AMP dose used ($P = 0.0012$ and $P = 0.0345$, respectively). These differences not only demonstrate the potentiating effect induced on the rabbit CC response when

L-Arginine was combined to AMP but also the existence of a clear synergistic effect. In fact, the sole addition of the relaxing effect induced by the two substances used separately was not sufficient to explain the increase of the AMP response in the presence of L-Arginine.

Discussion

The multifaceted nature of FSD is clearly distinct from that of the male [10] and generally regarded as being much more complex [11,12], involving a multitude of different factors such as the status of sex steroid hormones (testosterone, estrogens), critical in the maintenance of tissue structure and function, several central and peripheral reflex mechanisms (pelvic, hypogastric, pudendal nerves, lumbosacral spinal cord, thalamus, hypothalamus and at least five brain nuclei with their related neurotransmitters: norepinephrine, serotonin, dopamine, histamine, opioids and gamma-aminobutyric acid), various specific vasculogenic mediators (mainly vasoactive intestinal peptide, neuropeptide Y and NO) and moreover, psychological aspects. Amid this wide variety of key factors, the clitoris engorgement with blood due to sexual excitement plays a crucial functional role during intercourse in provoking orgasm [9,13] or at least pleasure and is essential to the maintenance of a normal sexual condition in female mammals.

No immunohistochemical or morphological differences have been reported between the penis
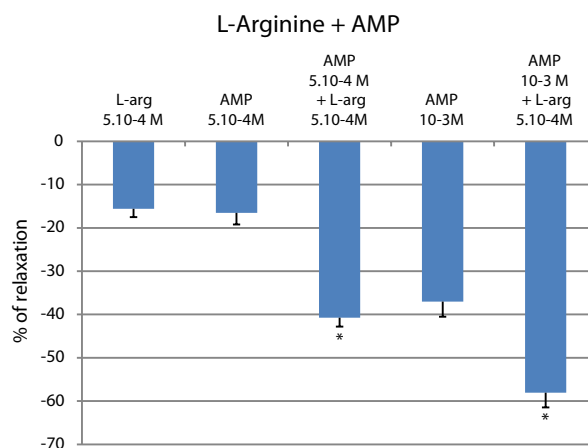
L-Arginine + AMP

Figure 4 AMP (5.10^{-4} and 10^{-3} M)/L-Arginine (5.10^{-4} M) association-induced relaxation on corpus cavernosum precontracted with phenylephrine. Vertical bars indicate SEM, (n = 12). AMP = adenosine monophosphate; SEM = standard error of the mean

and the clitoris in humans [3,8] with the exception of the subalbugineal layer [13,14]. In men, this area contains a rich venous plexus that is crushed during erection resulting in penile rigidity. The absence of this specific area in the clitoris limits the effects of the CC blood filling, which only leads to a tumescence. Therefore, safe drug helpers can be used, not only to induce this tumescence when required but also to ensure its maintenance during intercourse, which even makes much more sense in women with sexual arousal disorders than in men.

The female New Zealand rabbit is considered a reliable animal model to study clitoral engorgement and has been shown to characterize the mammal female sexual arousal response [7,15]. The existence of a clitoral adrenergic tone and the implication of the NO pathway, as a key mediating factor in clitoris smooth muscle relaxation, have also been well established [9,15–17]. In many respects, the overall vascular mechanisms that engorge the female erectile tissue are close to those already identified in the male erectile tissue. As a consequence, similar vasoactive medications could theoretically be used for both sexes. However, to date, subsequent experimental data have not been as conclusive as originally anticipated by the theory.

Several medications have been studied for their vasoactive effects on the female genitalia from which only two have emerged, estrogens [18] and phosphodiesterase type 5 inhibitors (iPDE5). Estrogens, whether or not combined with progestin therapy, can be used orally or topically. However, because the decision to use hormone therapy (HT) during menopause is made by each woman and her physician after weighing the benefits and possible risks of breast, endometrial, ovarian cancers, including other types of cancer (the lung for example), and not least the risk of cardiovascular diseases [19], it is clear that in many cases, FSAD alone does not justify the use of HT. In contrast to their use in men, iPED5 has failed to demonstrate consistent efficacy in women [20,21] except in those individuals with depression treated by selective serotonin reuptake inhibitors [22]. On the whole, iPDE5 has produced mixed preclinical results and equivocal human clinical results that did not encourage the Federal Drug Administration to approve this class of products for FSAD.

The most interesting data to date have been provided by a topically applied formulation of 0.4% alprostadil [23]. However, this prostaglandin E1 cream has not yet been approved; moreover, some women or their partners dislike the use of topical treatments for intercourse. Thus, there is a need for alternatives and possibly oral treatments. To our knowledge, there are no previously reported studies that have assessed the potential effect on the rabbit clitoral tissue of AMP alone or a combination of AMP with an NO donor. This includes findings based on our previous studies in the rabbit and human male [24].

In the present work, we demonstrated that (i) AMP significantly relaxes the CC of the female New Zealand White rabbit, however, comparatively not as much as it relaxed the male New Zealand White rabbit CC at identical concentrations (25% in female vs. 42% in male [2]); (ii) L-Arginine at 10–4 M was twice as potent on the female than on the male CC strips (16% vs. 8%); and (iii) the combination of AMP with L-Arginine potentiated each of other substance and induced a strong synergistic effect on the female CC strips (41% at AMP 5.10–4 M and 58% at AMP 10–3 M), which is approximately within the same range as the one observed on male rabbit CC strips (43% at AMP 5.10–4 M and 61% at AMP 10–3 M).

These results suggest that many similarities exist between both sexes in response to NO donors and AMP or their combination when these substances are tested on rabbit erectile tissue. It also indicates that very close biochemical mechanisms, although not to the same degree, are probably involved in the engorgement of the penis and the clitoris of the New Zealand White rabbit. However, there are also some differences: as opposed to what was observed in the male rabbit CC strip experiment, the effect of AMP on the rabbit clitoral tissue was linked to the NO pathway because L-NNA strongly inhibited the relaxation induced by AMP.

AMP is considered as an intermediary substance formed during the body's process of creating energy in the form of adenosine triphosphate (ATP). However, it does not activate P2 receptors [25]. Under physiological conditions, AMP behaves as a natural "adenosine precursor" that is ultimately converted into adenosine by ecto-5′-nucleotidases on the extracellular surface of all cells (due to this enzyme which has a very high affinity for AMP). Therefore, it is primarily seen as an agonist of adenosine/P1 receptors. Until the year 2012, there was no known specific AMP receptor in mammals. GPR80/GPR99, a P2Y receptor originally classified as an adenosine and AMP receptor [26], has since been discounted

[27,28]. A recent work using a novel cell-based assay to visualize adenosine receptor activation in real time associated with full inhibition of ecto-5′-nucleotidases activity could determine that AMP is in fact a direct A1 receptor agonist [29]. However, under normal conditions, ecto-5′-nucleotidases are not inhibited to the extent that AMP mainly acts through adenosine and not as a direct activator of P1 receptors.

Curiously enough, while P1 adenosine receptors, in particular the A2a and A2b subtypes, are widely present in the male CC of human, rabbit, and many other species, to our knowledge and to date, no P1 adenosine receptors have been found in the clitoral tissue of mammals. In contrast, the presence of P2Y receptors that recognize ATP, adenosine diphosphate, uridine triphosphate, and uridine diphosphate but not AMP, has been identified in rabbits and humans [12,30] localized to endocervical and cervical glands, epithelium, and stratified squamous epithelium of the vagina with a function of lubrification (stimulation of mucin secretion and chloride efflux).

The mechanism by which AMP induces female CC relaxation and interferes with the NO pathway is still unclear. Results in the female rabbit are comparable with those observed in the male rabbit with a higher response to AMP in the latter case and a more important L-Arginine-induced stimulation of the NO pathway in the former case. AMP is known to mainly act in mammals through the stimulation of vascular adenosine receptors. Although adenosine A2a receptors are found in the endothelia and vascular smooth muscle cells of the rabbit [31], to date, they have not been described in the clitoral CC of this animal model. In fact, it appears that no research has ever been conducted to explore this specific point. Our results prompted us to do so and to go deeper into the action mechanism.

As regards the complexity of the phenomenon involved in female CC relaxation, effective vasoactive agent combinations such as those described in our study should be considered as an interesting approach for the treatment of FSD. In the case of AMP and its combination to L-Arginine, further basic research and preclinical testing are warranted.

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Conflict of Interest: None.

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