Influence of Dopamine on Rat Uterine Motility *in vitro*

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Abstract

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The effect of dopamine on spontaneous rhythmic contractions of isolated rat uterine muscles was examined. The experiments were carried out on 25 female Buffalo rats aged between 4-6 months. Myometrial tissue (1.5- 2 cm long) was set up in 4 isolated organ baths (20 ml) at 37 °C. After recording the pattern of myometrial tissue spontaneous motility: dopamine, domperidone, metoclopramide, clozapine, bromocriptine, prazosine, propranolol were added into the organ baths. The dose of the formulae was fixed in preliminary experiments. Myometrial strips incubated in the isolated organ baths had spontaneous motility for about 8 to 12 h. The spontaneous motility frequency amounted to about 1.15 ± 0.38 contractions per min. The contraction strength was on average 2.8 ± 0.45 grams.

The addition of dopamine into the incubation bath organ at a concentration of $1.3 \cdot 10^{-4}$ mol/L significantly reduced the strength (63%, $p < 0.001$, to the value of 1.04 ± 0.18 grams) and frequency (27%, $p < 0.01$, to the value 0.84 ± 0.21 per minute) of spontaneous rhythmic contractions. The addition of dopamine at a concentration of $2.6 \cdot 10^{-4}$ mol/L totally inhibited the uterine spontaneous contractions. The attempt at blocking dopaminergic receptors with metoclopramide, domperidone and clozapine did not prevent uterus atony caused by the addition of dopamine. Uterus contractility was not affected by activating dopaminergic receptors with bromocriptine D_1 and D_2 receptors agonist, and the obtained recording exhibited no statistically significant differences in comparison to the initial recording. Simultaneous blocking of α_1 adrenergic receptors with prazosine and β adrenergic receptors with propranolol counteracted uterus atony after the addition of dopamine to the incubation bath organ.

The results indicated a blocking influence of dopamine on uterus contractility in rats *in vitro* related to a concentration of dopamine and caused by activating adrenergic receptors with dopamine.

Dopamine receptors, bath, incubation, myometrium, contractility, atony, receptor agonist, in vitro, rat

Dopamine participates in regulating many biological processes. To a large extent it influences the central nervous system and regulates movement control, muscle tension as well as the secretion of prolactin and sex hormones. Disorders connected with dopamine biosynthesis in the central nervous system cause many diseases e.g. Parkinson's disease, chorea. Dopaminergic receptors are also found outside the nervous system e.g. pituitary gland, parathyroid glands, thyroid gland, pancreas, digestive tract and ductus deferens. Dopamine works not only through dopaminergic receptors but also through the adrenergic ones (A n u r a s 1981; H o v e n d a l et al. 1982; W ę g r z y n et al. 1988; W ę g r z y n et al. 1994; Z i ę b a et al. 1991). Dopamine influences organs in many ways, which depends on the organ being examined and an animal's species. Dopamine blocks the motility of both human and canine stomach as well as that of rumen, abomasal and small intestine in ruminants (B u e n o et al. 1983; C e b r a t et al. 1989; D e C a r l e and C r i s t e n s e n 1976; W ę g r z y n et al. 1988; W ę g r z y n et al. 1994; Z i ę b a et al. 1991). On the other hand, it activates the motility of human duodenum and colon (A n u r a s 1981; L a n f r a n c h i et al. 1979; M a r z i o et al. 1986).

Dopamine has also an activating effect on myometrium. According to Zięba (Z i ę b a and D e j n e k a 1996) dopamine administered intravenously to sheep sensitized by stilboestrol causes an increase in uterus myoelectrical activity. The research carried out by A r k i n s t a l l and J o n e s

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(1985) also confirms the presence of this catecholamine in the uterine smooth muscles of humans, rabbit, sheep, guinea pig and rat. According to Moustafa, activating the dopaminergic receptor with bromocriptine (a selective D_2-agonist and weak partial agonist at the D_1 receptor) in both *in vitro* and *in vivo* experiments causes an increase in myometrial contraction in the early stage of rat pregnancy (Moustafa et al. 1999; Clark and White 1987).

Because to our knowledge no data are available on myometrial contraction under the effect of dopamine, our research was aimed at the study of the influence of dopamine on this phenomenon in rats under *in vitro* conditions.

Materials and Methods

The experiments were carried out on 25 Buffalo clinically healthy female rats aged 4-6 months at the weight of 150-170 g. Rats were housed collectively (5-6 rats in the metal cages) at a temperature of approximately 22 °C with a daylight cycle and fed *ad libitum* the LSM balanced rodent-diet (firm Agropol S.J.). The experiments were carried out with the consent of the Ethical Committee (No. 60/04). The animals were subjected to halothane general anaesthesia and after death their uterus strips 1.5-2 cm long were taken for examination. The strips were placed in 4 automatic bath organs of 20 ml capacity in accordance with the procedure of Alvarez et al. (1988) and Daly et al. (1981). The Krebs-Henseleit buffer (NaCl – 118 mM; KCl – 4.7 mM; $CaCl_2$ – 2.5 mM; $MgSO_4$ – 1.6 mM; $NaHCO_3$ – 24.3 mM; KH_2PO_4 – 1.18 mM; glucose 5.6 mM) (Coruzzi et al. 1988) was used as an incubation environment. The incubation of strips was conducted at the temperature of 37 °C, loaded with 1 g and the oxygen and carbon dioxide gas mixture (95% O_2 and 5% CO_2) was added so that its pH remained within 7.3-7.5. Myometrial contractions were recorded by isometric transducers (Letica Scientific Instruments) connected to BridgeAmp amplifiers (ADInstruments, Australia) with 4 channel data acquisition system (PowerLab/400, ADInstruments) connected to a Macintosh computer. After a 30-min recording of spontaneous myometrial contraction activity the following were added into the bath organs: dopamine – D_1 and D_2 receptors agonist (Dopamine hydrochloride SIGMA) at a concentration of 2.6 mol/L$\cdot 10^{-4}$, domperidone - D_2 receptor antagonist (Domperidone SIGMA) at a concentration of $0.67\cdot 10^{-3}$ (Laduron and Leysen 1979, Lazareno and Nahorski 1982), metoclopramide - D_2 receptor antagonist (Metoclopramide hydrochloride SIGMA) at a concentration of $0.83\cdot 10^{-3}$ (Pero 1999), clozapine (Clozapine SIGMA) at a concentration of $0.76\cdot 10^{-3}$ mol/L, bromocriptine – D_2 and D_1 receptor agonist (Bromergon-Lek Polska) at a concentration of $0.94\cdot 10^{-3}$ mol/L, prazosine-α_1-adrenergic receptors antagonist (Prazosin HCL, USP grade - RBI) at a concentration of $0.59\cdot 10^{-4}$ mol/L, propranolol-β_1 and β_2 adrenergic receptors antagonist (Propranolol Polfa) at a concentration of $0.8\cdot 10^{-4}$ mol/L.

In order to determine the effective dose the substances were added to the incubation bath ranging from the lowest dilution to the highest one in the dose concentration system (that is without rinsing the bath between the subsequent additions of the doses to the bath). The lowest substance concentration that yielded an evident result on the recording was accepted as the experimental dose and was confirmed in a number of following experiments.

The frequency of contractions per min and the strength of contractions given in grams were taken into consideration while summarizing the obtained results.

The results were summarized in the Microsoft Excel 2000 and analyzed statistically using the Student's *t*-test and the analysis of variance (ANOVA).

Results

The uterus strips placed in the automatic bath organ exhibited periodical contractility with a frequency of 1.15 ± 0.38 contraction per min. The strength of the single contraction was on average 2.8 ± 0.45 g.

The addition of dopamine to the incubation bath organ at a concentration of $0.26\cdot 10^{-4}$ mol/L per bath organ did not cause any statistical differences in uterus motility, but at a concentration of $1.3\cdot 10^{-4}$ mol/L caused a decrease in both the frequency (27% ($p < 0.01$), to the value 0.84 ± 0.21 per minute) and strength (63% ($p < 0.001$) to 1.04 ± 0.18) (hypotonia), and a total lack in spontaneous contractility at a concentration of $2.6\cdot 10^{-4}$ mol/L (atony) (Figs 1, 2, 3).

The attempt at blocking D_1 dopaminergic receptors with metoclopramide, domperidone and clozapine did not prevent from uterus atony caused by the addition of dopamine (Figs 4, 5, 6). Uterus contractility was not affected by activating dopaminergic receptors with bromocriptine and the obtained recording exhibited no statistically significant differences in comparison to the initial recording. Simultaneous blocking of β_1 adrenergic receptors with prazosine, and β adrenergic receptors with propranolol counteracted uterus atony caused by the addition of dopamine to the incubation bath (Fig. 7).
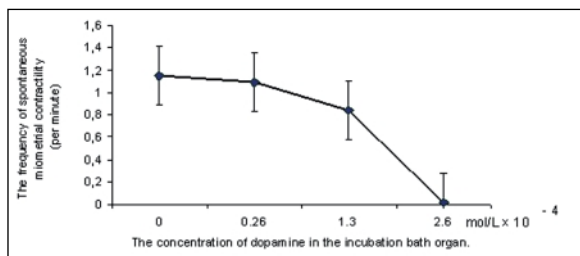
Fig. 1. The influence of dopamine concentration on the frequency of spontaneous uterine contractility in rats *in vitro*. An evident decrease in frequency and an increase in dopamine concentration.
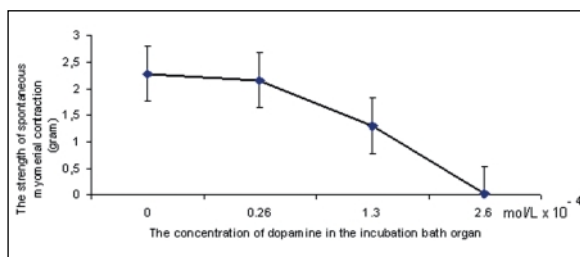

Fig. 2. The influence of dopamine concentration on the strength of spontaneous uterine contractility in rats *in vitro*. An evident decrease in strength and an increase in dopamine concentration.
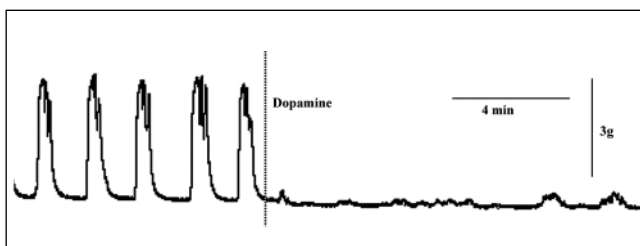

Fig. 3. The recording of uterus motility before and after the addition of dopamine at a concentration of 2,6 mol/L$\cdot 10^{-4}$. An evident decrease in motility of an isolated strip (atony). The addition time of dopamine was marked by the dotted line.
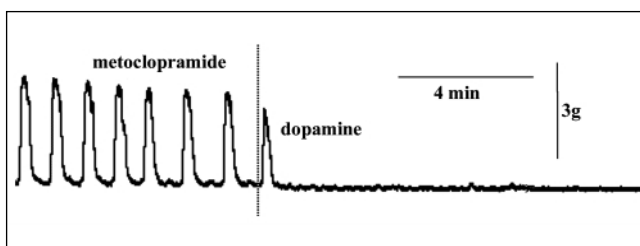

Fig. 4. The recording of myometrial contractility after blocking the D_2 dopaminergic receptor with metoclopramide and the addition of dopamine. An evident lack in the influence of metoclopramide on the blocking effect of dopamine on uterus contractility.
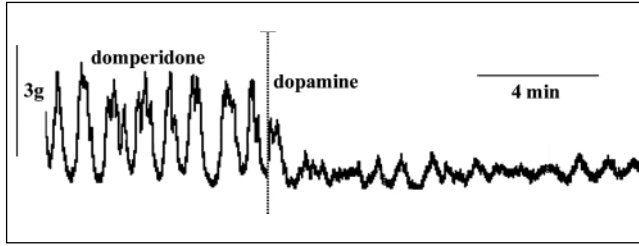
Fig. 5. The recording of myometrial contractility after blocking the D_2-dopaminergic receptor with domperidone and the addition of dopamine. An evident lack in the influence of domperidone on the blocking effect of dopamine on uterus contractility.
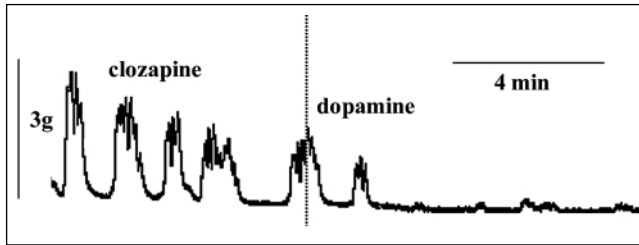

Fig. 6. The recording of myometrial contractility after blocking the D_1 and D_2-dopaminergic receptor with clozapine and the addition of dopamine. An evident lack in the influence of clozapine on the blocking effect of dopamine on uterus contractility.
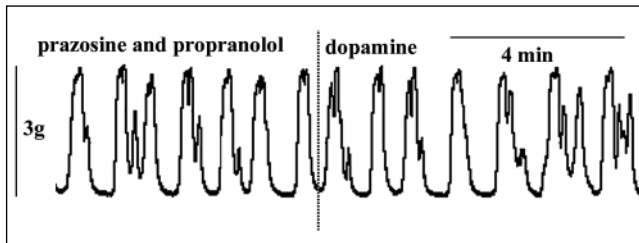

Fig. 7. The recording of myometrial contractility after blocking adrenergic receptors with prazosine and propranolol and the addition of dopamine. An evident lack in the influence of dopamine on spontaneous contractility after blocking adrenergic receptors.

Discussion

Administration of dopamine to animals causes different, often opposing reactions dependent both on the examined organs and the species. Dopamine influences organs not only through dopaminergic receptors but also through the adrenergic ones. According to Węgrzyn et al. (1994) dopamine blocks the motility of abomasum through D_2 dopaminergic and α-adrenergic receptors. Dopamine has also a blocking effect on the motility of ileum through α-adrenergic receptors (Zięba et al. 1991).

As far as uterine smooth muscles are concerned the presence of cell membrane receptors including the dopaminergic ones is strongly related to hormones, mainly oestrogens. Other authors also emphasize the importance of oestrogens in changing the concentration of different cell membrane receptors (Ayad et al. 1989; Gilbert et al. 1992; Massmann et

al. 1991; S h e l d r i c k and F l i n t 1985). A r k i n s t a l l and J o n e s (1985) indicated the presence of endogenous dopamine in uterus muscles, and F o r s b e r g et al. (1987) exhibited the influence of dopamine on rat uterus cervix contractility. According to Z i ę b a and D e j n e k a (1996) dopamine stimulates uterine myoelectrical activity in sheep sensitized by stilboestrol and its stimulating effect is not carried out by dopaminergic receptors. An increase in uterine pressure is caused by administration of dopamine to pregnant sheep (F i s h b u r n e et al. 1980). A similar increase in uterine contractility is noticed in women after the infusion of dopamine (U r b a n et al. 1982). An increase in uterus contractility is also noticed after the administration of bromocriptine (the agonist of dopaminergic receptors) to rats in early pregnancy (for the period of 11 days beginning from the 9th day) (M o u s t a f a et al. 1999).

Dopamine stimulates uterine contractility in rabbits, rats and women in early pregnancy (L a l and S h a r m a 1983; S i z o v 1992; U r b a n et al. 1982; E k e s b o et al. 1991). It blocks muscle relaxation effect caused by releasing relaxin during parturition (S i z o v 1992). The stimulating effect of dopamine and bromocriptine on uterus contractility *in vivo* is related to the fact that they increase the level of vasopressin (vasopressin stimulates uterus contractility), and decrease the concentration of PGI_2 (PGI_2 blocks uterus contractility) in the uterus (R o b i n s o n et al. 1982; H a u k s s o n et al. 1988; Ā g e e l et al. 1985).

The uterine strips placed in the automatic bath organ exhibited periodical contractility as confirmed by earlier experiments carried out by C z e r s k i et al. (2004) and other authors (E s t a n et al. 1988; P a r i j a et al. 2001). The addition of dopamine to the incubation bath organ at a concentration of $2.6 \cdot 10^{-4}$ mol/L resulted in atony of the isolated strip. Similarly, E s t a n et al. (1988) obtained atony of the isolated strips in rats after the addition of dopamine at a concentration of 10^{-7} to 10^{-4} mol/L. The decrease in uterine contractility was in direct proportion to the concentration of dopamine in the bath organ. On the contrary, L e c h n e r et al. (1996) obtained an increase in uterus activity *in vitro* after the addition of dopamine at a concentration of 10^{-6} mol/L. Blocking dopaminergic receptors with metoclopramide, domperidone and clozapine did not prevent from atony after the addition of dopamine. The obtained results indicated that the blocking effect of dopamine on uterus contractility was not carried out through dopaminergic receptors (E s t a n et al. 1988).

According to L e c h n e r et al. (2000) the addition of metoclopramide blocks spontaneous contractility of the isolated uterus strips removed from women during a caesarean section. The blocking effect of metoclopramide was not observed in our research. A different hormonal profile of a female (pregnancy) as well as a different species can change the influence of metoclopramide on spontaneous contractility. Dopamine can also influence organs through adrenergic receptors (e.g. W ę g r z y n et al. 1994; Z i ę b a et al. 1991).

Stimulating adrenergic receptors with selective agonists, except for α_2-adrenergic ones which has an activating effect, blocks uterus contractility in rats *in vitro* (C z e r s k i et al. 2004). In other animal species, for example in sheep, it can be different. Blocking adrenergic receptors with prazosine (the antagonist of α_1-adrenergic receptors) and with propranolol (the antagonist of both subtypes of β-adrenergic receptors) prevented from atony of uterus muscles after the addition of dopamine. Dopamine could cause uterine atony through stimulating adrenergic receptors. Similarly, E s t a n et al. (1988) also obtained a blocking effect of dopamine on uterus contractility carried out through β-adrenergic receptors. However, blocking β-adrenergic receptors with propranolol or blocking α_1-adrenergic ones with prazosine did not counteract uterine atony after the addition of dopamine. It was simultaneous blocking of both β-adrenergic and α_1-adrenergic receptors that counteracted the influence of dopamine on spontaneous contractility.

Účinek dopaminu na motilitu dělohy potkanů *in vitro*

Byl zkoumán účinek dopaminu na spontánní rytmické kontrakce izolovaných svalových buněk dělohy potkanů. Experimenty byly provedeny na 25 samicích potkanů Buffalo, ve věku 4-6 měsíců. Tkáň myometria (1,5-2 cm dlouhý proužek) byla vložena do 4 izolovaných orgánových lázní (20 ml) o 37 °C. Po zaznamenání rytmu spontánní motility tkáně myometria byly do orgánových lázní aplikovány: dopamin, domperidon, metoclopramid, clozapin, bromocriptin, prazosin a propranolol. Dávka látek byla stanovena v předběžných pokusech. Proužky myometria inkubované v izolovaných orgánových lázních vykazovaly spontánní motilitu po dobu cca 8 až 12 hodin. Frekvence spontánní motility se pohybovala kolem 1,15 ± 0,38 kontrakcí za minutu. Síla kontrakce byla průměrně 2,8 ± 0,45 gramů. Přidání dopaminu do orgánové inkubační lázně v koncentraci $1{,}3 \cdot 10^{-4}$ mol·l^{-1} signifikantně snížilo sílu (63%, $p < 0{,}001$, na hodnotu 1,04 ± 0,18 gramů) a frekvenci (27%, $p < 0{,}01$, na hodnotu 0,84 ± 0,21 za minutu) spontánních rytmických kontrakcí. Přidání dopaminu v koncentraci $2{,}6 \cdot 10^{-4}$ mol·l^{-1} zcela inhibovalo spontánní rytmické kontrakce dělohy. Pokus o blokování dopaminergních receptorů metoclopramidem, domperidonem a clozapinem nezabránilo atonii dělohy způsobené přidáním dopaminu. Kontraktilita dělohy nebyla ovlivněna aktivací dopaminergních receptorů bromokriptinem, agonistou D_1 a D_2 receptorů, a získané záznamy neprokázaly žádné statisticky významné rozdíly ve srovnání s výchozími. Simultánní blokování α_1 adrenergních receptorů prazosinem a β adrenergních receptorů propranololem působilo proti atonii dělohy po přidání dopaminu do inkubační orgánové lázně.

Výsledky ukazují blokující účinek dopaminu na kontraktilitu dělohy potkanů *in vitro* závislý na koncentraci dopaminu a způsobený aktivací adrenergních receptorů dopaminem.

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