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Evidence for a Negative Coupling between Dopamine D2 Receptors and PLC through a GTP-binding Protein, and phospholipase-A2 inhibition is devoid of effect on basal corticosteroid secretion in interrenal gland of frog *Rana ridibunda*

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ABSTRACT

Dopamine D2- receptors play a pivotal role in inhibiting aldosterone secretion from adrenocortical cells. It has been demonstrated that this effect is mediated through inhibition of phospholipids turnover, although coupling of these receptors to G protein in non-mammals vertebrate has never been investigated. The aim of the present study is to shed more light on this coupling, and to demonstrate the possible implication of phospholipase-A2 activity in dopamine-evoked inhibition of corticosteroid secretion. Our present data show that the inhibitory effect of dopamine and its D2 agonists, apomorphine and LY171555 (5 µM) on inositol phosphates (IP, IP2, IP3) formation in adrenal gland (interrenal) of frog Rana ridibunda was completely abolished by a 24 hours preincubation of interrenal slices with 1 µg/ml of pertussis toxin. this suggests that dopamine D2 receptors are coupled to phospholipase-C through GTP-binding protein sensitive to pertussis toxin. In addition, dopamine had no effect on cAMP formation. However, none one of the classical dopamine D2 antagonists, i.e metoclopramide, sulpiride, and domperidone appears to be able to reverse dopamine-induced inhibition of inositol phosphate production, thus suggesting that the pharmacological characteristics of D2 dopamine receptors in interrenal gland of the frog Rana ridibunda are different from those of the D2 subtypes previously described in mammals.

Moreover, we showed that a 20-min infusion of four consecutive and croissant doses of quinacrine, a phospholipase-A2 inhibitory factor, had no effect on basal corticosterone and aldosterone secretion from interrenal slices in perifusion system. These data support the conclusion that the activation of dopamine D2 receptors is coupled to inhibition of inositol phosphate formation (inhibition of phospholipase-C activity) and corticosteroid secretion in interrenal tissue of frog Rana ridibunda occurs through a GTP-binding protein (Gi protein), and that inhibition of phospholipase-A2 has no effect on corticosteroidogenesis.

Keywords: D2 receptors, GTP-binding protein (G protein), inositol phosphates, Interrenal gland, Phospholipase-A2, corticosteroidogenesis.

1. INTRODUCTION

An increasing amount of evidence suggests that dopamine is the major potent inhibitory factor on aldosterone secretion in both man and experimental animals (Brown et al., 1982, Lombardi et al., 1988, Cuche, 1988, for review see Missale et al., 1998). Activation of dopamine D2 receptors by dopamine reduces both basal and angiotensin-II-stimulated corticosteroid secretion from adrenocortical cells in man and mammals (Chang et al., 2007, Malchoff et al., 1986, Drake et al., 1984, Missale et al., 1988, for review see Missale et al., 1998, Pivonello et al., 2007, Spat et al., 2004). In addition, the administration of metoclopramide, a dopamine D2 receptor antagonist, to both rats and humans was shown to increase plasma aldosterone concentration without modifying any of the known stimulators of hormones release, an effect that was blocked by intravenous infusion of dopamine (Sowers et al., 1981, Noth et al., 1980, for review see Missale et al., 1998). These results suggest that dopamine regulates the secretion of aldosterone.

Moreover, based on pharmacological, molecular, and biochemical studies, five subtypes of dopamine receptors were characterized in the central nervous system, and peripheral tissues (for review see Missale et al., 1998,

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Pivonello et al., 2007). These receptors belong to the family of seven membrane domain G protein coupled receptors subtypes, named D1-D5. The analysis of dopamine receptors structure and function suggests the existence of two different groups of receptors : D1-like, including D1 and D5 receptors generally associated to a stimulatory function, and D2-like, including D2, D3 and D4 receptors, generally associated to an inhibitory function (for review see Missale et al., 1998, Pivonello et al., 2007, Ehrhart-Bornstein et al., 1998). In addition, two forms or subtypes of dopamine D2 receptor were later characterized and named D_{2S}, and D_{2L} (for review see Missale et al., 1998, Pivonello et al., 2007). In the adrenal cortex of man and rats, D1, D2, and D4 dopamine receptors were characterized, the D1 is coupled to adenylate cyclase stimulation, where D2, D3, and D4 are coupled to adenylate cyclase inhibition (Kwan-Dun et al., 2001, Aherne et al., 1997, Missale et al., 1986, Amenta et al., 1994). However, the inhibitory effect of dopamine appears as a complex mechanism because dopamine D2 receptors are known to regulate multiple transduction pathways. Indeed, many and recent studies conducted on adrenal cortex, pituitary lactotroph cells, and central nervous system indicate that the dopamine D2 receptors are negatively coupled to phosphoinositide turnover (Noth et al., 1980, Enjalbert et al., 1990, Simmonds et al., 1985), inhibition of cAMP formation (Missale et al., 1985), reduction of calcium mobilization (Malgaroli et al., 1987), activation of potassium channels (see for review Missale et al., 1998, Pivonello et al., 2007), modulation of Na^+/K^+ ATPase and Na^+/H^+ exchangers activities (for review see Missale et al., 1998, Pivonello et al., 2007). However, although it is now well accepted that dopamine inhibits aldosterone production from adrenal cortex through activation of D2 receptors, the mechanism of action of dopamine in corticosteroidogenesis has not been extensively studied in non-mammal vertebrate. We have previously shown that dopamine evokes a direct dose-dependent inhibition of basal corticosteroid production from the perifused frog interrenal gland (Morra et al., 1989, Morra et al., 1990). In this model, dopamine has a biphasic effect on phosphoinositide hydrolysis, e.i. a transient stimulation followed by a sustained inhibitory phase, and a decrease in diacylglycerol and arachidonic acid formation (Morra et al., 1991). Also arachidonic acid formation appears necessary to maintain the basal level of corticosteroid secretion in our model (Morra et al., 1989). In addition, Apomorphine inhibits phosphoinositide breakdown in frog interrenal tissue, suggesting that dopamine effect is mediated via activation of D2 receptors. Simultaneously, we showed that the stimulatory effect of dopamine on phosphoinositide hydrolysis and steroid secretion is mediated by activation of D1 receptors (Morra et al., 1992).

On the other hand, many studies have demonstrated that activation of dopamine D2 receptors-induced inhibition of phosphoinositide breakdown, cAMP formation , or others second messengers is sensitive to pertussis toxin treatment and many agents such as adenosine, calcitriol or carbachol (for review see Missale et al., 1998, Pivonello et al., 2007). In the present study, we have investigated the coupling of frog interrenal dopamine D2 receptors to GTP-binding protein. In addition, using an inhibitory factor of phospholipase-A2, we examined the influence of phospholipase-A2 on the basal level of corticosteroid production from perifused interrenal slices of frog.

MATERIALS AND METHODS

Animals

Adult male frogs (*Rana ridibunda*) of 40-50 g body weight were obtained from a commercial source. The animals were housed in a temperature-controlled room (8±1°C) under running water, on a 12 h light/dark schedule (lights on from 6:00 A.M. to 6:00 P.M.), for at least 1 week before use. To limit possible variations of steroid biosynthesis attributable to circadian rhythms, all animals were killed between 7:30 A.M. and 8:30 A.M.

(^{3}H) myo-inositol labeling

frogs were decapitated and the interrenal (adrenal) glands were dissected free of kidney tissue, sliced and preincubated at 24C° for 15 min in L15 medium (pH 7.4) complemented with 2mM $CaCl_2$, 0.2mg/ml glucose and 1% each of kanamycin and antibiotic-antimycotic solutions. Then, the interrenal slices were incubated at 24C° for 7h in 200µl fresh medium containing 100µl (^{3}H)-myo-inositol. At the end of the incubation period, the radioactive medium was discarded and the tissue was rinsed six times over a 30-min period with L15 medium containing 1mM nonradioactive myo-inositol. Thereafter, tissue slices were transferred into test tubes (six slices per tube) and incubated with 125µl L15 medium containing 10mM LiCl for 20 min before secretagogues

addition. This incubation period is necessary to block the activity of myo-inositol-1- phosphatase (Morra et al., 1991). Drugs to be tested were added in 125µl L15 medium for the times indicated in the figures and tables. Pertussis toxin (1µg/ml) was added 17h before the beginning of incorporation of (^{3}H)-myo-inositol. After incubation, the reaction was stopped by adding 250µl ice-cold 20% trichloroacetic acid solution. Then, the tissues were homogenized using a manual glass potter tube and centrifuged (13000g at 4Cº) for 5 min. The supernatant was separated and stored at -20Cº until (^{3}H)-inositol phosphate analysis. The pellet was suspended in 200µl EDTA until DNA determination.

Determination of Inositol Phosphates (Insp) Production

(^{3}H) inositol phosphates were separated by anion exchange chromatography on Dowex-1 columns (1x2 cm) as previously described (Morra et al., 1991, Morra et al., 1992). Elution of inositol monophosphate (IP), inositol biphosphate (IP2), and inositol triphosphate (IP3) was achieved with 30, 90, and 500 mM HCl respectively. The radioactivity found in the IP_1, IP_2, and IP_3 fractions was counted in a 1217 Rack Beta spectrometer (60% efficiency).

cAMP Measurement

The interrenal glands were quickly removed, and sliced into pieces of 2 mm length and preincubated at 24Cº for 15 min in 2 ml L15 medium (pH 7.4). Slices were then rinsed twice with 1 ml medium and incubated at 24Cº for 20 min in 150µl fresh medium containing 0.1mM IBMX. Thereafter, dopamine agonists or other tested substances were added in 150µl L15 medium for the time selected, and the tissue was incubated as previously described (Morra et al., 1992). The reaction was stopped by adding 150µl ice-cold 5% perchloric acid. Then, the tissues were homogenized and centrifuged at 13000g for 5 min at 4Cº. The supernatant containing cAMP was separated and stored at -20Cº. The pellets were suspended in 100µl EDTA (10mM) solution to prevent DNAase activity until DNA assay.

Before cAMP determination, the supernatant was neutralized using 100µl $KHCO_3$ (1M) and centrifuged at 13000g for 2 min at 4Cº. The supernatant was separated and diluted (1;1) with 0.05M acetate buffer (pH 5.5).The concentration of cAMP was determined in 100µl aliquot of each sample using a radioimmunoassay kit (Amersham).

DNA Measurement

Determination of DNA was performed as previously mentioned (Morra et al., 1991, Morra et al., 1992). Briefly, a 20µl aliquot from each sample was mixed with 2 ml buffer (7.4) consisting of 10 ml Tris base, 1mM EDTA, and 0.1M NaCl, containing compound H33258 at a final concentration of 0.1µg/ml. Fluorescence was read at 458nm in a TKO100 Mini-Flourometer (Hoefer Scientific Instruments, San Francisco, CA,USA) standardized with a 100 ng DNA solution.

Perifusion Technique

Details of perifusion system have previously been described (Leboulenger et al., 1978). Briefly, sliconized columns (0.9x12cm) limited by Teflon piston were repacked with 500µl of Bio-Gel P2 suspension (200-400 mesh;Bio-Rad laboratories, Richmad, CA).

Interrenal tissues were removed free of kidneys, dissected into slices and incubated in 5ml L15 medium (PH 7.4) for 15 min at 24Cº. Slices were then transferred into a new test tube containing 10 ml of L 15 medium in the presence of pertussis toxin (1µg/ml) and incubated at 24Cº for 17h. During this prolonged incubation the medium was renewed twice and gassed with 95% O2, 5% CO2. Thereafter, the tissues were rinsed twice in L15 medium free of pertussis toxin, transferred and layered into the columns between several 2-mm beds of Bio-Gel. The perifusion chambers were then continuously supplied with either Ringer`s solution alone or with tested substances dissolved in the Ringer's solution at a constant flow rate (200µl/min) and temperature (24Cº).

Secretion was allowed to stabilize for 2h before any substances were tested. The effluent perifusate from each column was stored frozen until corticosteroid assay.

Corticosteroid Radioimmunoassay

Corticosterone and aldosterone concentrations were directly determined in 100 µl aliquots of each fraction without prior extraction, using specific radioimmunoassay as previously described (Leboulenger et al., 1982). The working ranges of the assays were 20-5000pg for corticosterone and 7-640pg for aldosterone. None of the test substances interfered in the corticosterone and aldosterone assays.

Statistical Analysis

Corticosteroid concentrations were calculated from standard curves linearized by mean of the logit-log

transformation. Each perifusion pattern was established as the mean profile of corticosteroid production (± S.E.M) calculated over five to six independent experiments. For each perifusion profile, corticosterone and aldosterone levels were expressed as a percentage of the basal level value calculated as the mean of the eight consecutive fractions (40min) just preceding the infusion of each secretagogue. The Mann-Whitney test was used to determine the statistical significance of steroid responses to dopamine agonist administration.

For inositol phosphate and cAMP assays, results were expressed as the mean ±S.E.M of at last 5 independent experiments. Student`*t*-test was used to evaluate the statistical significance of the differences between control and experimental values.

RESULTS

1- Effect of pertussis toxin on dopaminergic agonists-induced decrease on (^{3}H) IPs formation

As illustrated in table (1), preincubation of interrenal slices with pertussis toxin (1 µg/ml) prevents apomorphine and LY171555-evoked inhibition of inositol phosphate production.

Table 1: Effect of pertussis toxin on dopaminergic agonists-induced inhibition of (^{3}H) IPx production. ^{3}H-myo-inositol prelabeled adrenal slices were exposed for 10 min to dopamine agonists (50 µM). Pertussis toxin (PTx, 1 µg/ml) was added one hour before dopamine addition. Data are the mean ± SEM of five independent experiments. *p<0.01 compared to control, N.S: non significantly different from control.

(^{3}H) inositol phosphates (cpm /µg DNA)

	(^{3}H)IP	**(^{3}H) IP2**	**(^{3}H) IP3**
control	840 ± 40	120 ± 9	40 ± 4
Dopamine	402 ± 30*****	58 ± 3*****	18 ±1.5*****
LY171555	414 ± 28*****	58 ± 3*****	19 ± 1.2*****
apomorphine	430 ± 20*****	60 ± 5*****	21 ± 2*****
Dopamine+PTx	810 ± 35 **N.S**	115 ± 8 **N.S**	41 ± 2 **N.S**
LY171555+ PTx	801 ± 29 **N.S**	112 ± 7 **N.S**	37 ± 3 **N.S**

2- Effect of dopamine antagonists on dopamine-evoked inhibition of (^{3}H)-inositol phosphate production

As shown in table (2), none of the classical dopamine antagonists used in our experiments, metoclopramide, sulpiride and domperidone, was able to abolish dopamine-induced inhibition of inositol phosphate formation in interrenal slices.

Table 2: Effect of dopamine antagonists on dopamine -induced inhibition of (^{3}H)IPx production. ^{3}H-myo-inositol prelabeled adrenal slices were exposed for 10 min to dopamine (50 µM). Metoclopramide, sulpiride, or domperidone (50 µM) were added 20 min before dopamine supply. Data are the mean ± SEM of five independent experiments. *p<0.01 compared to control.

(^{3}H) inositol phosphates (cpm /µg DNA)

	(^{3}H)IP	(^{3}H) IP2	(^{3}H) IP3
control	920 ± 34	140 ± 10	60 ± 4
Dopamine	430 ± 21*****	65 ± 4*****	26 ± 1.2*****
Dopamine + metoclopramide	452 ± 22*****	54 ± 3*****	22 ± 1.5*****
Dopamine + sulpiride	465 ± 28*****	61 ± 6*****	28 ± 2*****
Dopamine + Domperidone	448 ± 20*****	60 ± 4*****	25 ± 2*****

3- Effect of Dopamine on cAMP Formation

To verify whether, in our model, dopamine D2 receptors are coupled to the adenylate cyclase system, we investigated the effect of dopamine D2 agonists, apomorphine and LY171555 on cAMP formation. Table (3) demonstrates that 5µM forskolin induced a threefold increase in cAMP level. In contrast, exposure of interrenal segments to dopamine and the D2-dopaminergic agonists, LY171555 or apomorphine had no effect on cAMP generation.

Table 3: Effect of dopamine and dopaminergic agonists on cAMP formation by interrenal slices. Values are mean ± SEM of 10 determinations. Adrenal slices were preincubated in L15 medium containing 100 µM of IBMx for a period of 30 min, then treated for 5 min without (control) or with dopamine agonists (50 µM) or

with forskolin (5 µM) as mentioned in "Materials and Methods". N.S: non significantly different from control

Substances tested	cAMP (pmol /µg DNA)	n
Control	4.50 ± 0.14	10
Dopamine	4.7± 0.30 $^{N.S}$	10
Apomorphine	4.2 ± 0.16 $^{N.S}$	10
LY171555	4.9 ± 0.25 $^{N.S}$	10
Forskolin	12.50 ± 0.70	10

4- Effect of PLA2 Inhibition on Basal Level of Corticosteroid Release

To determine whether, on frog adrenocortical cells, the inhibition of PLA2 activity affects corticosteroid secretion, we have tested the effect of quinacrine, a PLA2 inhibitor on steroid release. Figure (1) shows that a 20-min infusion of three consecutive doses of quinacrine (5µM) had no effect on basal corticosterone and aldosterone secretion.

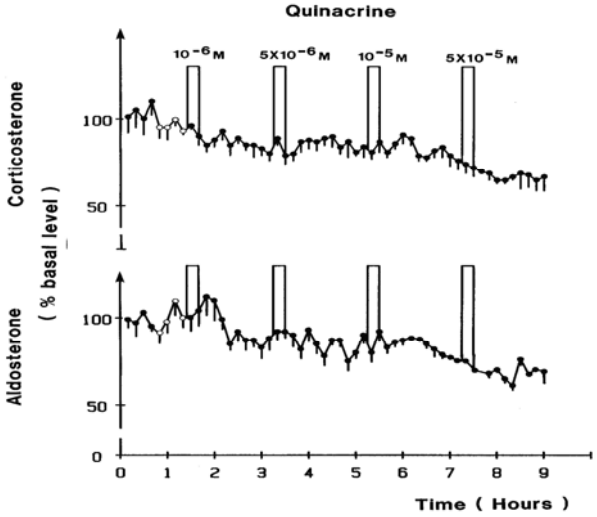

Figure1. Effect of four consecutive and croissant doses of quinacrine, a phospholipase-A2 inhibitor, on the secretion of corticosterone (A) and aldosterone (B) by perifused frog interrenal slices. The pulses of quinacrine (20 min each) were given at 90-min intervals. The profiles represent the mean ±SEM secretion pattern of five independent perifusion experiments. Mean basal levels of corticosterone and aldosterone in these experiments were 360 ± 24 and 150 ± 14 fmol/interrenal/min respectively.

DISCUSSION

The present data demonstrate that dopamine D2 receptor agonists-evoked inhibition of inositol phosphate production in frog adrenocortical cells are completely abolished by preincubation of adrenal slices with pertussis toxin. This observation suggests that these receptors are negatively coupled to phospholipase-C through GTP-binding protein. Moreover, the inhibitory effect of dopamine agonists on inositol phosphate formation was insensitive to dopamine D2-receptor antagonists metoclopramide, sulpiride, or domperidone. In addition, dopamine had no effect on cAMP formation, and that quinacrine, a phospholipase-A2 inhibitor factor, had no effect on basal level of corticosteroids release from perifused interrenal slices.

Our data demonstrate that pertussis toxin pretreatment of interrenal slices (adrenal gland) completely abolished dopamine-induced inhibition of inositol phosphate production, and that pertussis toxin blocked the effect of the two dopamine D2 agonists, apomorphine and LY 171555-induced decrease in phosphoinositide formation, suggesting that the D2 dopamine receptor in frog adrenal gland is coupled to GTP-binding protein (G_i protein).This

result correlates well with our previous demonstration which showed that preincubation of frog interrenal slices with pertussis toxin completely prevent dopamine-induced inhibition of corticosterone and aldosterone release by perifused interrenal slices of frog (Morra et al., 1991). Taken together, our data represent the first demonstration that the D2 dopamine receptor in interrenal gland of a non-mammal vertebrate is coupled to G protein. In accordance with these results, the recent studies conducted in adrenal cortex of man and other mammals showed that dopamine receptors belong to the superfamily of seven transmembrane domain coupled G protein (for review see Ehrhart-Bornstein et al., 1998, Missale et al., 1998, Pivonello et al., 2007). The coupling of D2 dopamine receptor to G protein was also demonstrated in rat lactotrophic pituitary cells where dopamine inhibits prolactin secretion (Enjalbert et al., 1983, 1990, Liu et al., 1994, see for review Missale et al., 1998).

However, we observed in the present study that the inhibitory effect of dopamine and dopamine D2 agonists on inositol phosphate production was not blocked by any of the classical D2 receptor antagonists, metoclopramide, sulpiride, or domperidone. The lack of effect of the D2 antagonists on dopamine-induced inhibition of inositol phosphate formation indicates that dopamine D2 receptor in interrenal gland of frog *Rana ridibunda* is not similar to that in man and other mammal species. This also is in accordance with our previous results showing that the classical D2 dopamine receptor antagonists were unable to block dopamine-evoked inhibition of corticosterone and aldosterone secretion from interrenal gland of the frog *Rana ridibunda* (Morra et al., 1992, see for review Sicard et al., 2008).

On the other hand, previous studies conducted in adrenal cortex of rats (Enjalbert et al., 1983, 1990), in the pituitary lactotrophic cells of man and rat (Liu et al., 1994, De Camilli et al., 1979, Mc Donald et al., 1984, for review see Pivonello et al., 2007), and in the rat striatum (Onali et al., 1985) have shown that the activation of dopamine D2 receptor can inhibit adenylate cyclase activity and thus decreases basal level of cAMP formation. In contrast, several studies demonstrated that, in zona glomerulosa of bovine adrenal gland and other mammal species, dopamine had no effect on basal cAMP formation (Stern et al., 1986, Bevilacqua et al., 1982, for review see Missale et al., 1998, Pivonello et al., 2007), thus supporting the present data showing that in interrenal gland of frog *Rana ridibunda*, dopamine is devoid of effect on basal cAMP production, and supporting our previous results demonstrated that dopamine D2 receptor is coupled to phospholipase-C (Morra et al., 1991).

Moreover, since the activation of dopamine receptors in central nervous system and peripheral tissues appears also to be able to activate other mechanisms of signal transduction pathways including the modulation of phospholipase-A2 activity, an enzyme able to catalyze directly the formation of arachidonic acid from membrane phospholipids (PI), and also to modulate the activity of phospholipase-D, an enzyme able to catalyze the hydrolysis of phosphatidylcholine to phosphatidic acid and choline (Senogles, 2000, 2003, for review see Pivonello et al., 2007, Sicard et al., 2008), we thus tested the possible implication of phospholipase-A2 in dopamine-evoked inhibition of corticosteroid release from interrenal gland. Our data which demonstrate that the infusion of croissant doses of quinacrine, a phospholipase-A2 inhibitor is devoid of effect on corticosterone and aldosterone secretion from interrenal slices in perifusion indicate that the inhibition of phospholipase-A2 activity, and thus the release of arachidonic acid by this pathway, have no effect on basal secretion of corticosteroids. Nevertheless, we observed that the infusion of quinacrine to perifused interrenal slices induced a weak and gradual diminution on the spontaneous release of corticosteroids. The significance of this decrease is unknown and remains questionable. However, since our present data show that the administration of repeated and croissant doses of quinacrine to perifused interrenal slices does not induce a rapid reversible and reproducible inhibition of corticosteroid secretion suggests that phospholipase-A2 have no effect on corticosteroid release, and that dopamine inhibits corticosteroidogenesis independently of phospholipases-A2 activity.

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إثبات ارتباط سلبي بين المستقبلات الدوبامينية D2 وإنزيم محال الدهون المفسفرة-C بوساطة بروتين رابط للجوانوسين ثلاثي الفوسفات، وتثبيط محال الدهون المفسفرة-A2 لا يؤثر على الإفراز القاعدي للستيرويدات القشرية من الغدة الكظرية للضفدع رانا ريديبيوندا

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ملخص

تؤدي المستقبلات الدوبامينية D2 دوراً هاماً في تثبيط إفراز هرمون الألدوسترون من خلايا قشرة الكظر. وأكدت نتائج الأبحاث أن هذا التأثير يمر عبر تثبيط تحولات الدهون المفسفرة، لكن ارتباط هذه المستقبلات إلى بروتين G في الفقاريات غير الثديية لم تُبحث أبداً. إن الهدف من هذه الدراسة هو تسليط مزيد من الضوء على هذا الارتباط، وبيان دور محتمل لإنزيم محال الدهون المفسفرة - A2 في التأثير المثبط للدوبامين على إفراز الستيرويدات القشرية. تُظهر نتائج الدراسة أن الحضن المسبق لقطع الغدة الكظرية، لمدة 24 ساعة، مع سم جراثيم السعال الديكي بتركيز 1 ميكرو مول، يكبح تماماً التأثير المثبط للدوبامين والشادات الدوبامينية من النمط D2، مثل الأبومورفين، وLY171555 (5 ميكرو مول)، على اصطناع مركبات الإينوسايتول فوسفات (IP1، IP2، IP3) في خلايا الغدة الكظرية للضفدع رانا ريديبيوندا، وهذا يؤكد أن المستقبلات الدوبامينية -D2 ترتبط إلى إنزيم محال الدهون المفسفرة-C عبر بروتين رابط للجوانوسين ثلاثي الفوسفات حساس لسم جراثيم السعال الديكي. إضافة لذلك ليس للدوبامين أي تأثير على اصطناع الجوانوسين أحادي الفسفات الحلقي.

على أية حال، لم يتمكن أي من الحاصرات الدوبامينية D2 الكلاسيكية مثل الميتوكلوبراميد، والسلبيريد، والدمبيريدون، من كبح التأثير المثبط للدوبامين على اصطناع مركبات الاينوسايتول فوسفات، مما يفترض أن المستقبلات الدوبامينية D2 في خلايا الغدة الكظرية للضفدع رانا ريديبيوندا تختلف بخصائصها الصيدلانية عن المستقبلات الدوبامينية D2 التي وصفت سابقاً في الثدييات.

إضافة إلى ذلك، تبين نتائج دراستنا أن حقن أربع جرعات متتالية ومتزايدة (لمدة 20 دقيقة لكل منها) من مادة الكيناكراين، وهو مثبط لنشاط إنزيم محال الدهون المفسفرة-A2، لم يؤثر في الإفراز القاعدي لهرموني الكورتيكوسترون والألدوسترون من الغدة الكظرية في تجارب الحضن الخلوي.

تخلص هذه النتائج إلى أن تفعيل المستقبلات الدوبامينية D2 الذي يؤدي إلى تثبيط اصطناع مركبات الاينوسايتول فوسفات، ومن ثم تثبيط إفراز الستيرويدات القشرية من خلايا الغدة الكظرية للضفدع إنما يتم عبر مستقبلات D2 المرتبطة إلى إنزيم محال الدهون المفسفرة-C بوساطة بروتين رابط للجوانوسين ثلاثي الفوسفات (البروتين Gi)، وأن تثبيط إنزيم محال الدهون المفسفرة-A2 لا يؤثر في إنتاج الهرمونات الستيرويدية.

الكلمات الدالة: المستقبلات D2، البروتين G، اينوسايتول فوسفات، الغدة الكظرية، إنزيم محال الدهون المفسفرة-A2، الستيرويدات القشرية.

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