

Behaviour of neural and renal (Na⁺, K⁺)-ATPase to dopamine and amphetamine

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(Reçu le 17/12/1996 ; Accepté le 23/02/1997)

تفاعل ATPase المستحضرة من الجهاز العصبي والكلية مع الدوبامين والأمفيتامين

تمت دراسة مفعول الدوبامين والأمفيتامين على نشاط ATPase في البصلة الشمية والكلية لدى الفأر. تفاعل هذا الأنزيم مع المادتين يختلف كثيرا إذا كانت مستحضرة من البصلة الشمية أو الكلية. ويبدو هذا الاختلاف أكثر حين تتراوح نسب المادتين بين M⁻⁵¹⁰ و M⁻⁴¹. في هذه الحالة لاحظنا حصرا ضعيفا في الكلية، أما في البصلة الشمية فإن المادتين قد أثرتا بشكل واضح على نشاط الأنزيم. وهكذا كان أقصى هذا الأثر 102% بالنسبة للدوبامين و 25% بالنسبة للأمفيتامين. نفس المفعول لوحظ حين دراسة pNPPase، حيث أن تفاعل هذا الأنزيم مع المادتين كان مختلفا حسب العضو الذي استحضرت منه. نلاحظ أن معالجة مستحضر البصلة الشمية بمنق (التريتون) يؤدي إلى اختفاء تام لأثر المادتين على الأنزيم. هذه المعطيات تدل على أن الدوبامين و الأمفيتامين

(M⁻⁵¹⁰ إلى M⁻⁴¹⁰) لانتثران بصفة مباشرة على ATPase في الجهاز العصبي، وقد يكون أثرهما ناتجا عن تفاعلها مع غشاء الخلية التي تشكل المحيط الذي يوجد فيه الأنزيم في الجهاز العصبي.

الكلمات المفتاحية : ATPase - البصلة الشمية - الكلية - الدوبامين - الأمفيتامين - المحيط الغشائي

Comportements de la (Na⁺, K⁺)-ATPase nerveuse et rénale vis-à-vis de la dopamine et de l'amphétamine

L'action *in vitro* de la dopamine (DA) et de l'amphétamine (AMPH) a été étudiée sur la (Na⁺, K⁺)-ATPase du bulbe olfactif et du rein du rat. La réponse de cette enzyme aux deux drogues dépend énormément de l'organe d'origine de la préparation enzymatique surtout pour des doses comprises entre 10⁻⁵ et 10⁻⁴M. Sur cette gamme de concentration, au niveau rénal, ces deux agents ont exercé une faible inhibition non significative; au niveau bulbaire, ils ont induit, en revanche, une stimulation significative dont le maximum est de 102% (DA) et de 25% (AMPH). La K⁺-pNPPase a présenté le même comportement que la (Na⁺, K⁺)-ATPase. Celui-ci dépend, également, de l'organe d'origine de la préparation enzymatique. Cet effet stimulateur disparaît lorsque la préparation enzymatique d'origine nerveuse est traitée par le triton X 100 (0,03%). Les résultats de ce travail montrent que la DA et l'AMPH (10⁻⁵ à 10⁻⁴M) n'ont pas d'effet direct sur la (Na⁺, K⁺)-ATPase nerveuse. l'effet stimulateur "apparent" pourrait être dû à une interaction entre ces drogues et l'environnement membranaire de l'enzyme au niveau de la cellule nerveuse.

Mots clés : (Na⁺, K⁺)-ATPase - Bulbe olfactif - Rein - Dopamine - Amphétamine - Environnement membranaire

Behaviour of neural and renal (Na⁺, K⁺)-ATPase to dopamine and amphetamine

The action of dopamine (DA) and amphetamine (AMPH) on the (Na⁺, K⁺)-ATPase from the rat olfactory bulb and kidney was studied *in vitro*. The effects of the drugs depended greatly on the organ from which the enzyme was extracted, particularly in the concentration range 10⁻⁵ to 10⁻⁴ M. In this range the two agents produced a weak (non-significant) inhibition of activity in the kidney enzyme, while producing a very significant stimulation (maximum 102% for DA and 25% for AMPH) in the olfactory bulb enzyme. The same effects were also observed on the K⁺-pNPPase, which showed a behaviour dependent on the organ from which it was extracted. The stimulatory effect disappeared once the neural enzyme preparation was treated with triton X100 (0.03%). These results are consistent with the theory that the stimulatory effect is attributable to an interaction between the drugs and the membrane environment of the enzyme in nerve cells.

Key words : (Na⁺, K⁺)-ATPase - Olfactory bulb - Kidney - Dopamine - Amphetamine- Membrane environment

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INTRODUCTION

The (Na⁺, K⁺)-ATPase (sodium pump) plays a major role in cellular function. It ensures the transport of Na⁺ and K⁺ ions against their electrochemical potential gradients across animal cell membranes. In the kidney, it plays a role in the membrane transport of glucose (Kinne *et al.*, 1975), amino acids (Evers *et al.*, 1976), citrate and lactate (Kinne and Schwartz, 1978). In the brain, it can intervene in the release of neurotransmitters (Mc Millen, 1983).

The involvement of the neural sodium pump in the release of catecholamine and, notably, of dopamine (DA) under the influence of amphetamine (AMPH) (Mc Millen, 1983) suggests that this drug may act on the activity of (Na⁺, K⁺)-ATPase. In fact, some studies have already described the action *in vitro* of different drugs (phenobarbital, ouabain...) on this activity (Deliconstantinos 1983; Lichtstein *et al.*, 1985). The response is often biphasic: stimulation at weak doses (10⁻⁷ to 10⁻⁵M) and inhibition at strong doses (10⁻³ to 10⁻²M) of these drugs. While the inhibitory effect at strong doses is widely accepted, the stimulation at weak doses is not well characterised. With the aim of determining how weak doses of pharmacological agents might induce stimulation, the present paper studies the actions *in vitro* of AMPH and DA on membrane preparations obtained from total homogenization of the olfactory bulb of the rat.

Given the fact that some studies (Jensen & Ottolenghi 1976) have shown that the activity of (Na⁺, K⁺)-ATPase is very sensitive to perturbations of the lipid bilayer, we have also studied the effects of treatment with a detergent. In these conditions, we have shown (unpublished) that triton X 100 (TX 100) (the detergent used in the present work) at certain doses does not denature the enzyme protein but does perturb the lipid environment of the enzyme.

The activity of (Na⁺, K⁺)-ATPase follows a cycle with phosphorylation of the enzyme being followed by dephosphorylation. The dephosphorylation step is coupled to a K⁺-dependent phosphatase activity (Robinson, 1973). This is usually evaluated by studying the K⁺-p-nitrophenylphosphatase (K⁺-pNPPase) activity (Swann, 1984). The actions of AMPH and DA will therefore also be studied on this enzyme.

Finally, the behaviour of (Na⁺, K⁺)-ATPase from the kidney will also be subjected to the same

procedures to provide a comparison with the behaviour of the neuronal preparation.

MATERIAL & METHODS

1. Enzymatic preparation

The animals used in this experiment were adult female albinos wistar rats (*Ratus norvegicus*), weighing about 300g. After decapitation, the forebrain, including both olfactory bulbs or kidney were quickly removed and kept in ice cold 0,25 M sucrose solution.

For the olfactory bulb, homogenization was done directly in a Potter-Elvehjen grinder type AA. For the kidney, disaggregation was initiated in an Ultra-Turrax homogenizer and finished in a Potter-Elvehjen. For both tissues, the homogenization was done in a solution of 0,25 M sucrose. The volume was chosen to give a convenient concentration of proteins for the enzymatic reactions to occur. The homogenates were placed in closed tubes, frozen at -18°C and kept for 24 hours in the freezer before biochemical evaluation. Protein content was measured using the method of Lowry *et al.* (1951). Bovine serum albumin was used as a standard.

2. Enzymatic activity measurement

• (Na⁺, K⁺)-ATPase

(Na⁺, K⁺)-ATPase activity was estimated by measuring the quantity of inorganic phosphate (Pi) produced during an incubation (with or without drugs) for 15 minutes in an agitation bath thermostated at 37°C in the presence of excess ATP substrate. The method for measuring Pi was that of Baginski *et al.* (1967). The medium used for the estimation of total ATPase activity contained: Tris-HCl buffer, pH 7,4: 100 mM; MgCl₂: 3 mM; NaCl: 100 mM; KCl: 30 mM. Mg⁺⁺-ATPase activity was measured in a medium containing: Tris-HCl buffer, pH 7,4: 100 mM; MgCl₂: 3 mM; NaCl: 130 mM; ouabain: 1 mM. Ouabain-sensitive ATPase activity ((Na⁺, K⁺)-ATPase) was obtained by subtracting Mg⁺⁺-ATPase from total ATPase activity.

• K⁺-pNPPase

K⁺-pNPPase activity was estimated by measuring the quantity of p-nitrophenol (pNP) produced during an incubation (with or without drugs) for 15

minutes in an agitation bath thermostated at 37°C in the presence of p-nitrophenylphosphate (pNPP). The pNP produced following the transformation of pNPP reacted immediately with NaOH (0,2M) to produce a yellow colour. The medium used for the estimation of total pNPPase activity contained: Tris-HCl buffer, pH 7,4: 100 mM; MgCl₂: 5 mM; KCl: 30 mM. Mg⁺⁺-pNPPase activity was measured in a medium containing: Tris-HCl buffer, pH 7,4: 100 mM; MgCl₂: 5 mM; KCl : 30 mM; ouabain: 1 mM. Ouabain-sensitive pNPPase activity (K⁺-pNPPase) was obtained by subtracting Mg⁺⁺-pNPPase from total pNPPase activity.

3. Triton X 100 treatment of enzymatic preparations

Detergent treatment of enzymatic preparations was carried out as follows: Triton X 100 (TX100) was dissolved in water at 0,06 per cent concentration. 0,5 ml of this solution was added to 0,5 ml of enzyme suspension. Activity of 0,1 ml of the enzyme-detergent mixture (0,03% of TX100) was determined immediately following preincubation at 0°C for 15 min.

4. Data analyses

All results are means \pm S.E.M of five separate homogenate preparations and they are expressed

as the pourcentage of activity measured in the absence of drug (control, 100%). The statistical significance of differences between control and treatments was determined using Student's t-test. N.S. : non significant ; *: 0,001 < p < 0,05 ; **: p < 0,001

RESULTS

1. Protein levels and enzyme activities in the olfactory bulb and kidney

In table 1 the results are presented as a comparison between the two tissues and show :

- The level of proteins in the olfactory bulb is around 70% of that in the kidney.
- The basal activities (Mg⁺⁺-ATPase and Mg⁺⁺-pNPPase) are 2 to 3 times higher in the kidney.
- iii) The stimulated activities (Na⁺, K⁺)-ATPase and K⁺-pNPPase show nearly the same level in the two tissues.
- iv) TX 100 at 0.03% produces in general an activation of the different enzymes, ranging from 30 to 80%.

2. Action of increasing doses of amphetamine and dopamine on the activities of Mg⁺⁺-ATPase and Mg⁺⁺-pNPPase *in vitro*

The basal levels of activity did not change significantly under the influence of AMPH and DA. Tables 2 & 3 summarize the behaviour of Mg⁺⁺-ATPase and Mg⁺⁺-pNPPase in response to two

Table 1. Protein content and specific activities of ATPases and pNPPases in olfactory bulb and kidney

Tissue	Protein ^a	Mg ⁺⁺ -ATPase ^b		(Na ⁺ K ⁺)ATPase ^b		Mg ⁺⁺ -pNPPase ^c		K ⁺ pNPPase ^c	
		CP ^d	TX100	CP ^d	TX100	CP ^d	TX100	CP ^d	TX100
Olfactory bulb	124 ± 4 (26)	9,23 ± 0,27 (7)	8,63 ± 0,31 (4)	5,51 ± 0,30 (7)	9,36 ± 0,80 (4)	1,22 ± 0,08 (6)	1,65 ± 0,03 (4)	1,08 ± 0,20 (6)	1,38 ± 0,10 (4)
Kidney	167 ± 5 (15)	18,40 ± 0,60 (5)	16,20 ± 0,50 (4)	4,40 ± 1,10 (5)	8,00 ± 1,00 (4)	3,27 ± 0,10 (4)	4,17 ± 0,10 (4)	0,84 ± 0,20 (4)	1,13 ± 0,20 (4)

Each value represents the mean \pm S.E. of the number of separate homogenate preparations given in parentheses.

a : mg/g de tissu ; b : mmoles Pi/mg prot/h ; c : mmoles pNP/mg prot/h ; d : Control preparation

Table 2. Effects of DA and AMPH (3x10⁻⁵ M and 10⁻²M) on Mg⁺⁺-ATPase activity in olfactory bulb and kidney

	Without drugs	DA 30 mM	AMPH 30 mM	DA 10000 mM	AMPH 10000mM
Olfactory bulb	100%	95 ± 4 N.S.	100 ± 6 N.S.	71 ± 3*	72,5 ± 3*
Kidney	100%	102 ± 4 N.S.	103 ± 4 N.S.	79 ± 3*	69,5 ± 3*

Table 3. Effects of DA and AMPH (3x10⁻⁵ and 10⁻²M) on Mg⁺⁺-pNPPase activity in olfactory bulb and kidney

	Without drugs	DA 30 mM	AMPH 30 mM	DA 10000 mM	AMPH10000 mM
Olfactory bulb	100%	101 ± 5 N.S.	105,7 ± 5 N.S.	74 ± 3*	86 ± 3*
Kidney	100%	102,5 ± 6 N.S.	104 ± 5 N.S.	90 ± 4*	90,5 ± 3*

dose levels that induced the largest effects on the (Na⁺, K⁺)-ATPase and K⁺ pNPPase activity levels. At 3×10^{-5} M neither of the two drugs had a significant effect. By contrast, at 10^{-2} M both compounds had a significant inhibitory effect (10 to 30%).

3. Action of increasing doses of amphetamine and dopamine on the activities of (Na⁺, K⁺)-ATPase and K⁺-pNPPase *in vitro*

Figures 1 and 2 show that (Na⁺, K⁺)-ATPase and K⁺-pNPPase behave in a similar way under the

influence of AMPH and DA and that the behaviour of both depends on the tissue from which they were extracted. In the case of the kidney (right hand panels), both agents produce a dose-dependent inhibition, which is more striking in the case of AMPH. In the case of the olfactory bulb (left hand panels), both produce a biphasic effects on both enzymes : i.e. a very significant stimulation which reaches maximal effects at 80-102% for DA (at 10^{-4} M) and 20-25% for AMPH (at 3×10^{-5} M). Only at 10^{-2} M do these agents produce a significant inhibition (between 50 and 80%) of both enzymes.

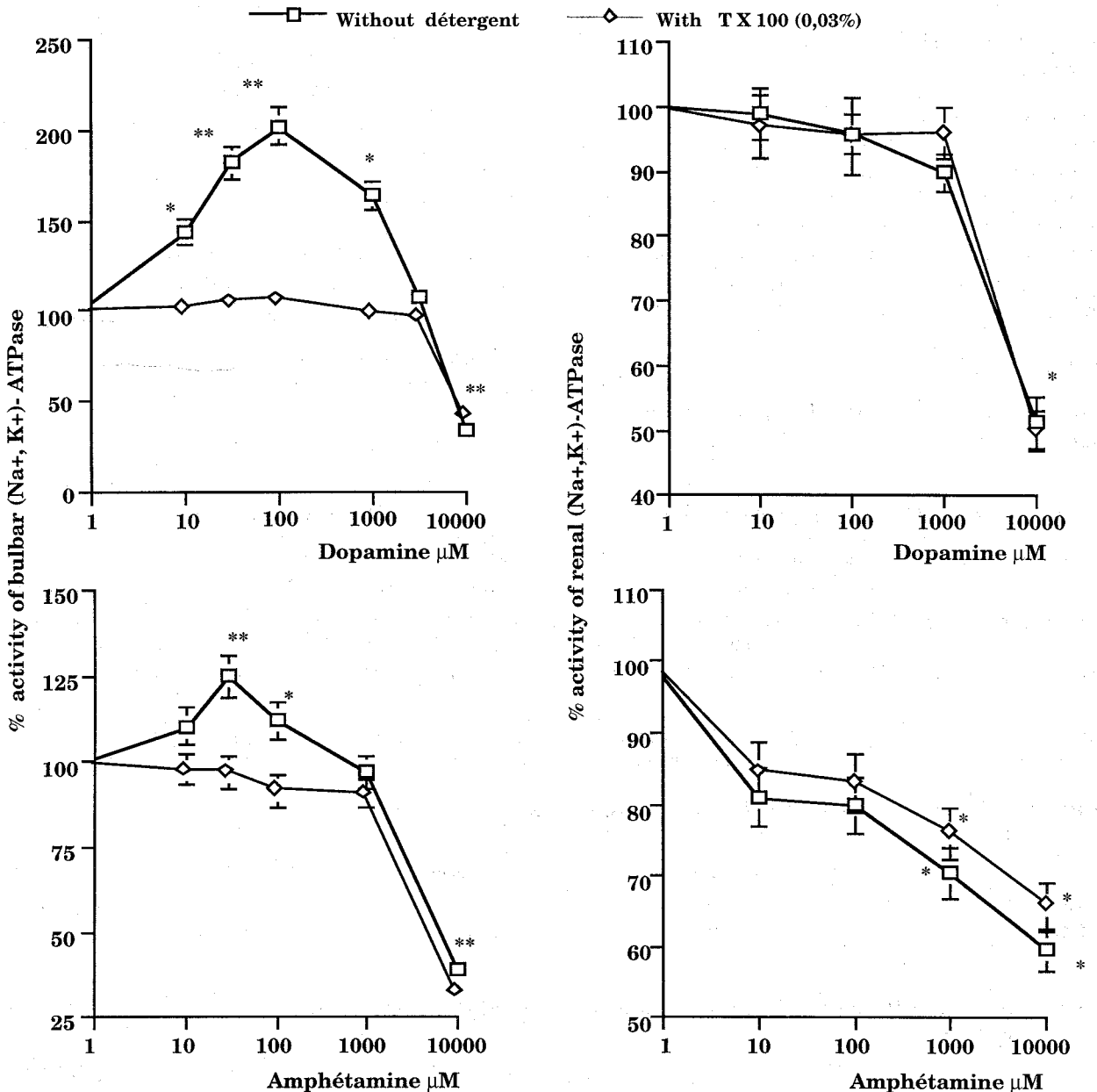


Figure 1. Effects of various concentrations of DA and AMPH on (Na⁺, K⁺)-ATPase activity in olfactory bulb and kidney

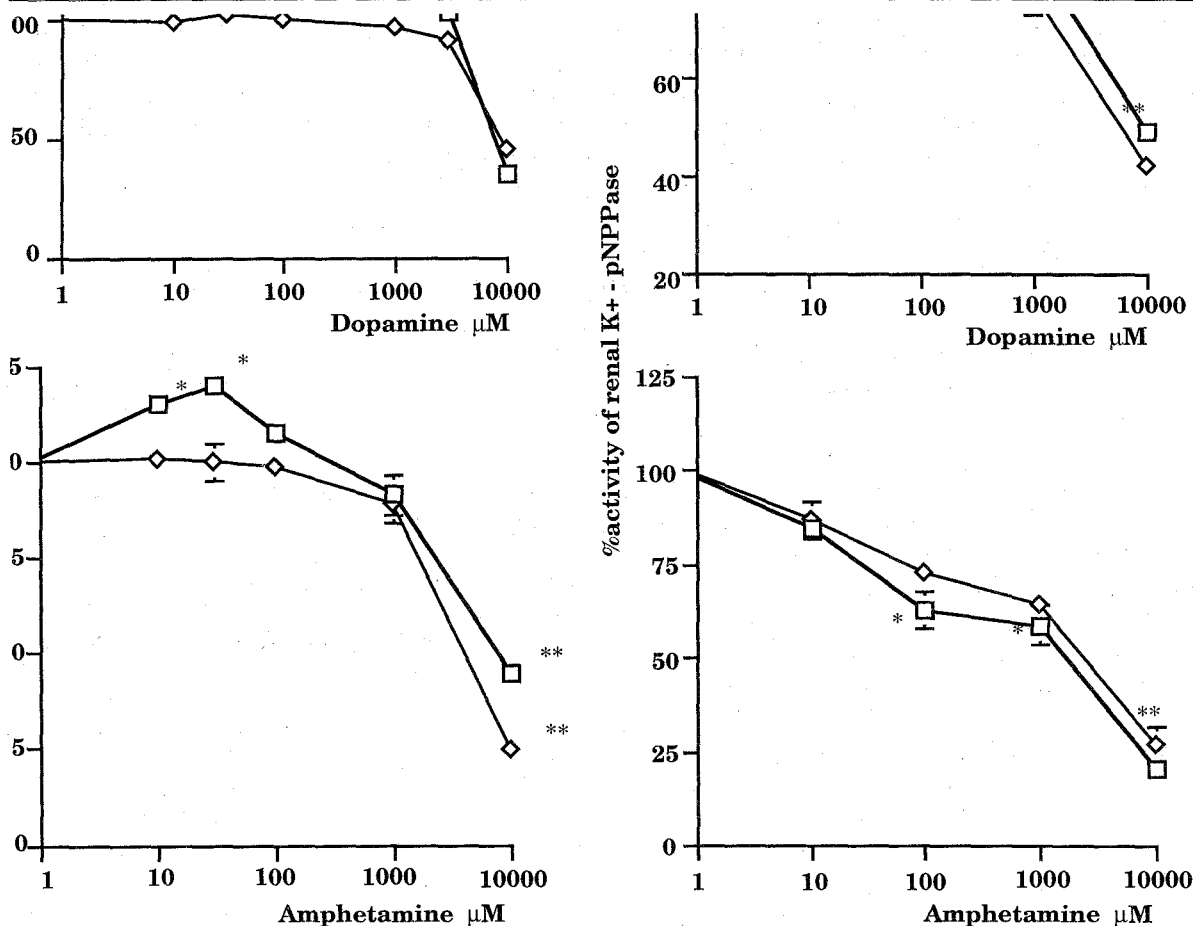


Figure 2. Effects of various concentrations of DA and AMPH on K⁺-pNPPase activity in olfactory bulb and kidney

It is clear that TX 100 (0.03%) has no influence on the effects of either drug on the renal enzymes. By contrast, it significantly reduced and even abolished the stimulatory effect of weak doses on enzymes from the olfactory bulb. For the two drugs, the remaining effect (when it existed) did not exceed 10% and was not significant.

DISCUSSION

Regulation of the (Na⁺ K⁺)-ATPase by different factors has been demonstrated by a variety of hormones (Johnson *et al.*, 1986; Gick & Ismail-

Beigi, 1990). neurotransmitters (Hernandez-R, 1992; Vizi & Oberfrank, 1992) and other endogenous factors (Hamlyn *et al.*, 1991). DA has been shown to inhibit the (Na⁺, K⁺)-ATPase in the kidney (Bertorello & Aperia, 1990), brain (Bertorello *et al.*, 1990) and rat tail arteries (Rashed & Songy-Mize, 1995).

In this study we investigated the regulation of the neural (Na⁺, K⁺)-ATPase activity by DA and AMPH, and identified some of the mechanisms involved in the stimulatory effect obtained between 10⁻⁵ and 10⁻⁴M of these drugs.

The results reported here show that the response of the (Na⁺, K⁺)-ATPase to DA and AMPH depends greatly on the organ from which the enzyme preparation is made. Our results show that between 10⁻⁵ and 10⁻⁴ M the neural (Na⁺, K⁺)-ATPase is much more sensitive to DA than to AMPH. The stimulatory effect of the former can reach an increase 102%, while the latter produces only 25%.

By contrast, in the kidney, the stimulatory effect is absent. This difference between neural and renal enzymes is consistent with the theory that catecholamines uptake (DA) in neurons involves transportation of amines from the synaptic cleft to the neuronal cytoplasm, which is facilitated by (Na⁺, K⁺)-ATPase. This difference between neural and renal enzymes could be explained by the existence of two isomers of (Na⁺, K⁺)-ATPase: α (+) is predominant in the central nervous system (CNS) while α exists in kidney (Swadlow, 1979).

The structural and physico-chemical properties of these two isomers are, doubtless, responsible for different responses to pharmacological agents. These isoforms can be characterized by isoform-specific pharmacologic properties (Shyjan *et al.*, 1990; Jewell & Lingrel, 1991), suggesting that there may be isoform-specific functions in cell of the CNS and kidney.

Our results show that this stimulatory effect disappears when the CNS enzyme preparation is treated with TX 100 at 0.03%. By contrast, this treatment has no effect on the kidney enzyme preparation. These results are consistent with those of Matsuda & Iwata (1986) who show that the form α (+) is more sensitive to the action of phospholipase than the form α . Our results also show that a similar, organ-dependent, difference exists for the K⁺-pNPPase.

These results, as a whole, show that, at weak concentrations (10⁻⁵ to 10⁻⁴M), DA and AMPH do not act directly on the neural (Na⁺, K⁺)-ATPase. The apparent stimulatory effect could rather be due to a non-specific interaction between these pharmacological agents and the lipid environment of the (Na⁺, K⁺)-ATPase (Peters, 1977).

In physiological conditions, this environment exerts an inhibitory effect on the enzyme molecule (Deliconstantinos, 1983; Lichtstein *et al.*, 1985).

The augmentation of activity by weak concentrations of drug is therefore better represented as a removal of these environmental constraints: a form of "disinhibition" similar to that postulated already for the low dose stimulatory effects of cardiac glycosides (Lichtstein *et al.*, 1985).

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