

THE STATE OF DOPAMINE, SEROTONIN, ADRENAL AND GLUCOCORTICOID RECEPTORS IN CHRONIC FLUORIDE INTOXICATION

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ABSTRACT

The aim of the study was to assess the state of receptor binding parameters under the action of sodium fluoride on the organism under model conditions for the intoxication formation.

Material and methods. The experimental part of the work was performed on 16 white rats of the Wistar population. Internal organs and tissues were subjected to the study of the state of receptor binding parameters of labeled C1, C2 serotonin, $\alpha 1$, $\alpha 2$, β -adreno, D2-dopamine and glucocorticoid type II receptor agonists and antagonists in various regions of the brain and liver. The functional activity of the receptors was assessed by affinity and the number of binding sites and ligands.

Results. The study of the effect of sodium fluoride on the kinetic characteristics of the adrenoreceptors of the membrane fractions of liver cells and the cerebral cortex showed similar dynamic changes in the parameters of receptor binding of both $\alpha 1$ -adreno- and $\beta 1$ -adrenoreceptors. The effect of sodium fluoride was manifested in a decrease in the affinity of the radioligand for $\alpha 2$ -adrenoreceptors and an increase in the number

RÉSUMÉ

La condition des paramètres de liaison aux récepteurs de l'adrénaline, la dopamine, la sérotonine et glucocorticoïde dans les conditions de l'intoxication chronique au fluorure de sodium

Le but de la recherche était d'étudier l'état des paramètres de liaison au récepteur sous l'action du fluorure de sodium sur l'organisme dans des conditions modèles pour la formation d'une intoxication.

Matériel et méthodes. La partie expérimentale du travail a été réalisée sur 16 rats blancs de la population Wistar. Les organes et les tissus internes ont été soumis à l'étude de l'état des paramètres de liaison au récepteur des agonistes et antagonistes marqués des récepteurs C1, C2, $\alpha 1$, $\alpha 2$, β -adréno, D2-dopamine et glucocorticoïdes de type II, dans diverses régions du cerveau et du foie. L'activité fonctionnelle des récepteurs a été évaluée par l'affinité et le nombre de sites de liaison et de ligands.

Résultats. L'étude de l'effet du fluorure de sodium sur les caractéristiques cinétiques des récepteurs adrénergiques des fractions membranaires des cellules du

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of binding sites for this type of receptors. Similar dynamics of the kinetic characteristics of $\alpha 2$ -adrenoreceptors was found in the cerebellum. The number of binding sites for $\beta 1$ -adrenoreceptors increased, while their affinity for ligands decreased. In the brainstem, there was a decrease in the affinity of the D2-receptors for ligands and the number of binding sites. Under conditions of subtoxic effect of sodium fluoride on white rats, a decrease in the affinity of radioligands for C2-receptors and the number of their binding sites in the cerebral cortex was observed.

Conclusions. The results of the study show that under the conditions of the formation of fluoride intoxication, structural and functional disorders of the receptor apparatus occur, which confirms the leading role in the development of this pathology of the state of biological membranes. Changes in the kinetic characteristics of adrenaline ($\alpha 1$, $\alpha 2$, $\beta 1$), serotonin (C1, C2), dopamine (D2) and glucocorticoid receptors confirm the polytropic nature of the action of sodium fluoride on organs, systems and functions of the body.

Keywords: sodium fluoride, receptors, number of binding sites, equilibrium constant dissociation.

Abbreviations:

Cd = equilibrium constant dissociation
Bmax = number of binding sites
CNS = central nervous system
cAMP = cyclic adenosine monophosphate

INTRODUCTION

The study of the receptor apparatus of cellular structures has become one of the most important sections of molecular biology. It plays an extremely important role in understanding the mechanisms of homeostasis and pathogenesis of various diseases, intoxications, metabolic disorders in the assessment of hormonal regulation, immunobiological reactivity, the occurrence of remote consequences of the influence of harmful factors of the surrounding and industrial environment on the body^{1,2}. According to their purpose, receptors are sensors of the endocrine system, capable of distinguishing hormones from other chemical structures. Receptor structures bind a hormone for the time it takes for it to interact with various intermediaries through which the hormonal response is realized^{3,7}. Among the many chemical compounds, there are those that have the properties

of competitive binding to hormones, thereby disrupting the function of the receptor apparatus^{8,9}. foie et du cortex cérébral a montré des modifications dynamiques similaires des paramètres de liaison aux récepteurs des récepteurs adrénérgiques $\alpha 1$ -adrénérgiques et $\beta 1$. L'effet du fluorure de sodium s'est traduit par une diminution de l'affinité du radio-ligand pour les récepteurs $\alpha 2$ -adrénérgiques et une augmentation du nombre de sites de liaison pour ce type de récepteurs. Une dynamique similaire des caractéristiques cinétiques des récepteurs $\alpha 2$ -adrénérgiques a été observée dans le cervelet. Le nombre de sites de liaison des récepteurs $\beta 1$ -adrénérgiques a augmenté, tandis que leur affinité pour les ligands a diminué. Dans le tronc cérébral, il y avait une diminution de l'affinité des récepteurs D2 pour les ligands et du nombre de sites de liaison. Dans des conditions d'effet sous-toxique du fluorure de sodium sur des rats blancs, une diminution de l'affinité des radio-ligands pour les récepteurs C2 et du nombre de leurs sites de liaison dans le cortex cérébral a été observée.

Conclusions. Les résultats de l'étude montrent que, dans les conditions de formation d'une intoxication au fluorure, des troubles structurels et fonctionnels de l'appareil récepteur se manifestent, ce qui confirme le rôle de premier plan dans le développement de cette pathologie de l'état des membranes biologiques. Les modifications des caractéristiques cinétiques des récepteurs adrénaline ($\alpha 1$, $\alpha 2$, $\beta 1$), sérotonine (C1, C2), dopamine (D2) et glucocorticoïdes confirment le caractère polytropic de l'action du fluorure de sodium sur les organes, les systèmes et les fonctions du corps.

Mots-clés: fluorure de sodium, récepteurs, nombre de sites de liaison, dissociation à constante d'équilibre

of competitive binding to hormones, thereby disrupting the function of the receptor apparatus^{8,9}.

Researches of Seeman (1982) and Zhukov (1991) showed that almost all hormones probably have various ways to regulate metabolism by acting on receptors, intracellular mediation and the genome of cells, selectively suppressing or enhancing expression¹⁰. According to the generally accepted system, hormones penetrate from the bloodstream into the target cell either by free diffusion or by using special membrane systems that allow binding and transfer to the cell, where they are associated with cytoplasmic protein receptors¹¹. The resulting hormone receptor complex is activated and acquires an increased affinity for DNA and components of the nucleus. Subsequently, the activated hormone complex is translocated into the nucleus, where it induces early effects. Our previous studies have revealed a significant effect of sodium fluoride on the structure of membranes – changes in the ratio of phospholipid fractions, stimulation of

lipid peroxidation, a decrease in the activity of marker membrane-specific enzymes, stress function of the monooxygenase system and impaired tissue respiration and oxidative phosphorylation¹²⁻¹⁴.

THE OBJECTIVE WAS TO STUDY the state of receptor binding parameters under the action of sodium fluoride on the organism under model conditions for the intoxication formation.

MATERIALS AND METHODS

The experimental part of the work was performed on 16 white rats of the Wistar population, in the morning, on an empty stomach. For 1.5 months daily, the fasting sodium fluoride was orally administered at the rate of 20 mg/kg of body weight. At the end of the sub-acute experience, the animals were killed by decapitation. Internal organs and tissues were subjected to the study of the state of receptor binding parameters of labeled C1, C2 serotonin, α_1 , α_2 , β -adreno, D2-dopamine and glucocorticoid type II receptor agonists and antagonists in various regions of the brain and liver. The functional activity of the receptors was assessed by affinity and the number of binding sites and ligands. The method of radioligand receptor binding was used. The value of specific binding was determined by the difference between general and non-specific binding. The results were analysed

in the Scatchard coordinates. Kinetic characteristics were expressed in terms of Cd (equilibrium constant dissociation) and Bmax (number of binding sites), taking into account the methodological recommendations of P.V. Sergeev, N.L. Shimanovsky (1987) according to generally accepted methods.

RESULTS

The study of the effect of sodium fluoride on the kinetic characteristics of the adrenoreceptors of the membrane fractions of liver cells and the cerebral cortex showed similar dynamic changes in the parameters of receptor binding of both α_1 -adreno- and β_1 -adrenoreceptors. It should be noted that the affinity of radioligands for α_1 -adrenoreceptors decreased in the cortex and the number of their binding sites increased. A different picture of the kinetic characteristics was noted when evaluating β_1 -adrenoreceptors, for which the affinity for radioligands and the number of binding sites decreased. In the liver, the affinity of radioligands for α_1 -adrenoreceptors and the number of receptor binding sites decreased. The effect on β_1 -adrenoreceptors was characterized by an increase in the affinity of radioligands for receptors and a decrease in the number of their binding sites (Table 1).

In the next series of experiments, the kinetic parameters of α_2 -adrenoreceptors in the brain structure – the cortex, stem, cerebellum – were studied. The effect

Table 1. Effect of sodium fluoride on the kinetic characteristics of α_1 , β_1 – adrenoreceptors of membrane fractions of liver cells and cerebral cortex in the formation of fluoride intoxication. (Cd – nM, Bmax – pmol/mg protein)

| Groups of animals | Objects of study | | | | | | | |
|--------------------|-----------------------------|---------------------|------------------------------|---------------------|-----------------------------|---------------------|------------------------------|---------------------|
| | Liver | | | | Cerebral Cortex | | | |
| | β_1 – adrenoreceptors | | α_1 – adrenoreceptors | | β_1 – adrenoreceptors | | α_1 – adrenoreceptors | |
| | B_{max} | Cd | B_{max} | Cd | B_{max} | Cd | B_{max} | Cd |
| Control (n=7) | 0.27±0.003 | 4.30±0.18 | 0.78±0.0003 | 7.60±0.34 | 0.25±0.006 | 1.85±0.21 | 1.19±0.03 | 3.40±0.20 |
| Experimental (n=9) | 0.08±0.0002 P<0.05 | 2.40±0.17 P<0.05 | 0.48±0.0004 P<0.05 | 16.4±0.57 P<0.05 | 0.12±0.003 P<0.05 | 21.5±0.35 P<0.05 | 1.72±0.06 P<0.05 | 13.8±0.46 P<0.05 |

Table 2. The effect of sodium fluoride on the kinetic characteristics of the binding of 3H-rauvoxin in brain structures under the conditions of the formation of fluoride intoxication. (Cd- nM, Bmax – pmol/mg protein)

| Groups of animals | Objects of study | | | | | |
|--------------------|------------------------------|----------------------|------------------------------|----------------------|------------------------------|-----------------------|
| | Cerebellum | | Trunk | | Brain cortex | |
| | α_2 – adrenoreceptors | | α_2 – adrenoreceptors | | α_2 – adrenoreceptors | |
| | B_{max} | Cd | B_{max} | Cd | B_{max} | Cd |
| Control (n=7) | 19.6±0.40 | 0.82±0.004 | 21.4±0.43 | 0.52±0.03 | 25.4±0.9 | 0.33±0.002 |
| Experimental (n=9) | 27.5±0.60 P<0.05 | 0.54±0.003 P<0.05 | 32.6±1.15 P<0.05 | 0.46±0.002 P<0.05 | 42.6±2.3 P<0.05 | 0.80±0.0003 P<0.05 |

of sodium fluoride was manifested in a decrease in the affinity of the radioligand for $\alpha 2$ -adrenoreceptors and an increase in the number of binding sites for this type of receptors. Similar dynamics of the kinetic characteristics of $\alpha 2$ -adrenoreceptors was found in the cerebellum (Table 2). Analyzing the kinetic parameters of receptor binding in the medulla oblongata $\alpha 1$ -adreno-, $\alpha 2$ -adreno- and $\beta 1$ -adrenoreceptors, the increase in the affinity of radio-ligands for $\alpha 1$ -adrenergic receptors and a decrease in the number of binding sites for this type of receptors was established. In all cases, $\alpha 2$ -adrenoreceptor activity increased – the affinity of the number of radioligand binding sites increased.

As follows from the results shown in Table 3, the number of binding sites for $\beta 1$ -adrenoreceptors increased, while their affinity for ligands decreased. Determination of receptor binding parameters of dopamine receptors was carried out in the cerebral cortex, stem, and cerebellum. According to existing concepts, this type of receptor is divided into two types: D1 associated with adenylate cyclase and D2 – not associated with adenylate cyclase or involved in its inactivation¹⁵⁻¹⁶. Neuroleptics used in endocrine, neurological and psychiatric disorders are known to modify the activity of the D2-dopamine receptors. According to many authors, most of the toxic effects of dopamine agonists are mediated by D2 receptors¹⁷⁻¹⁹.

³H-spiperone was used as a labeled ligand, which has a high affinity for D2-dopamine receptors. The results of the experiments revealed a change in the kinetic parameters of the functional activity of this type of receptors, which consisted in a change in the affinity of radioligands and the number of their receptor binding sites. The directionality of these processes in different parts of the brain was ambiguous. An increase in receptor affinity for ligands and a decrease in the number of binding sites were observed in the cerebral cortex and cerebellum. In the brainstem, there was a decrease in the affinity of the D2-receptors for ligands and the number of binding sites (Table 4).

The established dissociation constants and kinetic characteristics of serotonin receptors of the first C1 and second C2 types showed a change in their functional activity in the cortex, stem, and cerebellum of the brain. The action of sodium fluoride was accompanied by an increase in the affinity of the ligand for C2-receptors in the cortex, stem, and a decrease in the cerebellum. The number of binding sites increased in the brainstem (Table 5). Under conditions of subtoxic effect of sodium fluoride on white rats, a decrease in the affinity of radioligands for C2-receptors and the number of their binding sites in the cerebral cortex was observed (Table 6).

Table 3. The effect of sodium fluoride on the adrenoreceptors kinetic characteristics of the medulla oblongata in conditions of the formation of fluoride intoxication. (Cd – nM, Bmax – pmol/mg protein)

| Groups of animals | Objects of study | | | | | |
|--------------------|---|-----------|---|-----------|--|------------|
| | medulla oblongata, α_1 - adrenoreceptors | | medulla oblongata, α_2 - adrenoreceptors | | medulla oblongata β_1 -adrenoreceptors | |
| | B_{max} | Cd | B_{max} | Cd | B_{max} | Cd |
| Control (n=7) | 1.50±0,16 | 8,2±0,14 | 0,05±0,0002 | 7,2±0,09 | 0,58±0,0002 | 1,25±0,015 |
| Experimental (n=9) | 0,75±0,06 | 5,10±0,22 | 0,12±0,003 | 4,60±0,07 | 0,804±0,0003 | 3,10±0,06 |
| | P<0,05 | P<0,05 | P<0,05 | P<0,05 | P<0,05 | P<0,05 |

Table 4. The effect of sodium fluoride on the binding parameters of ³H-spiperon by D2-dopamine receptors. (Cd – nM, Bmax – pmol/mg protein).

| Groups of animals | Objects of study | | | | | |
|--------------------|----------------------|------------|---------------------|--------------|--------------------------|------------|
| | Cortex, D2-receptors | | Trunk, D2-receptors | | Cerebellum, D2-receptors | |
| | B_{max} | Cd | B_{max} | Cd | B_{max} | Cd |
| Control (n=7) | 92.5±2.6 | 0.38±0.004 | 49.3±1.7 | 0.21 ± 0.015 | 81.4±1.50 | 0.41±0.003 |
| Experimental (n=9) | 69.5±2.6 | 0.17±0.002 | 42.4±1.16 | 0.34±0.0007 | 64.2±2.17 | 0.25±0.003 |
| | P<0.05 | P<0.05 | P<0.05 | P<0.05 | P<0.05 | P<0.05 |

It is well known that an important place in the regulation of metabolic processes and maintaining homeostasis belongs to glucocorticoid receptors and their sensors^{20,21}. When studying the state of type II glucocorticoid receptors, we used glucocorticoid dexamethasone, which, as is well known, does not interact with other types of glucocorticoid cytoplasmic receptors and transcortin. The results of radioligand binding of glucocorticoid receptors in the liver, cortex, stem, and cerebellum showed a similar direction, which was characterized by an increase in the number of this type of receptors in all organs (Table 7).

DISCUSSION

Analysis of the kinetic parameters of receptor binding shows that the affinity of radioligands for α 1-adrenoreceptors and the number of sites of their

binding decreases in the liver, the identical dynamics of the kinetic characteristics of β 1-adrenoreceptors was observed in the cerebral cortex. These data indicate that sodium fluoride has an antagonistic effect on the function of the receptor unit of hepatocytes and nerve cells. In the cerebral cortex, receptor affinity for ligands decreased and the number of α 1-adrenoreceptor binding sites increased, whereas receptor binding dynamics in the liver had a different direction, that is, the affinity of radioligands for receptors increased and the number of binding sites decreased.

According to modern concepts, the common property of α 2-adrenoreceptors is the induction of cAMP reduction through inhibition of adenylate cyclase²². Stimulation of α 2-adrenoreceptors may be accompanied by an increase in vagal influence, a decrease in the activity of the peripheral nervous

Table 5. The effect of sodium fluoride on the binding parameters of 3H-serotonin receptors of the first type (C1) in brain structures (Cd – nM, Bmax – pmol/mg protein).

| Groups of animals | Objects of study | | | | | |
|--------------------|----------------------------------|------------|---------------------------------|-----------|--------------------------------------|-----------|
| | Cortex, C _r receptors | | Trunk, C _r receptors | | Cerebellum, C _r receptors | |
| | B _{max} | Cd | B _{max} | Cd | B _{max} | Cd |
| Control (n=7) | 302.4±6.7 | 1.44±0.03 | 385.4±7.2 | 1.70±0.06 | 225.6±3.8 | 1.03±0.03 |
| Experimental (n=9) | 365.8±6.9 | 1.16±0.006 | 310.7±5.8 | 1.30±0.04 | 265.8±4.9 | 1.35±0.06 |
| | P<0.05 | P<0.05 | P<0.05 | P<0.05 | P<0.05 | P<0.05 |

Table 6. The effect of sodium fluoride on the binding parameters of 3H-spiperon receptors of the second type (C2) in brain structures (Cd – nM, Bmax – pmol/mg protein).

| Groups of animals | Objects of study | | | |
|--------------------|----------------------------------|-------------|---------------------------------|------------|
| | Cortex, C _r receptors | | Trunk, C _r receptors | |
| | B _{max} | Cd | B _{max} | Cd |
| Control (n=7) | 33.5±0.57 | 0.36±0.006 | 36.8±0.43 | 0.29±0.003 |
| Experimental (n=9) | 25.3±0.45 | 0.20±0.0015 | 26.5±0.42 | 0.14±0.002 |
| | P<0.05 | P<0.05 | P<0.05 | P<0.05 |

Table 7. The effect of sodium fluoride on glucocorticoid receptor type II (rmol/mg protein).

| Organs, used indicator | Group of animals, M±m | |
|---|-----------------------|-----------------------|
| | Control (n=7) | Experimental (n=9) |
| Liver, glucocorticoid receptors type II | 520.4±10.3 | 710.5±9.2 P<0.05 |
| Cerebellum, glucocorticoid receptors type II | 615.7±16.5 | 930.8±12.4 P<0.05 |
| Brain stem, glucocorticoid receptors type II | 850.6±22.4 | 2485±40,6 P<0.05 |
| Cerebral cortex, glucocorticoid receptors type II | 640.3±17.8 | 1702.6±50.4 P<0.05 |

system, arterial pressure, a violation of the secretory function of the gastrointestinal tract, etc.²³⁻²⁵

Of the known adrenergic mechanisms of the CNS one can suppose that in the cells of various brain regions due to the detected changes the following should be observed: disturbances on the part of phospholipid fractions, in particular, phosphatidylinositol, a decrease in the level of intracellular cAMP and a decrease in adenylate cyclase activity, which testify to a deep reorganization of cellular metabolism²⁶⁻²⁸.

The violation of the structural and functional state of dopamine D2-receptors as a result of a change in their dissociation constant and the number of binding sites should be expected to lead to the difficulty of neurotransmitter information transfer to intracellular structures and the realization of its function by dopamine²⁹.

The observed increase in the number of glucocorticoid receptors is probably associated with a powerful manifestation of the effect of nuclear translocation of this type of receptors in combination with steroids³⁰. It is possible that the prolonged effect of sodium fluoride on the change in the homeostatic level of the glucocorticoid hypothalamic-pituitary-adrenal system in animals leads to the involvement of type II glucocorticoid receptors and the genetic apparatus of the cell in the structurally metabolic process, which probably increases the body's resistance to harmful factors. The different level of type II glucocorticoid receptors in the animal studied tissues is determined, apparently, by the severity of their cellular metabolism.

CONCLUSIONS

Thus, the results of the study show that under the conditions of the formation of fluoride intoxication, structural and functional disorders of the receptor apparatus occur, which confirms the leading role in the development of this pathology of the state of biological membranes. Changes in the kinetic characteristics of adrenaline (α_1 , α_2 , β_1), serotonin (C1, C2), dopamine (D2) and glucocorticoid receptors confirm the polytropic nature of the action of sodium fluoride on organs, systems and functions of the body.

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Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article“

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law.“

„All institutional and national guidelines for the care and use of laboratory animals were followed“

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