

Role of endogenous opioid systems in alcohol reinforcement and dependence mechanisms

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Original article

SUMMARY

Biochemical and behavioral evidence indicate that the dopaminergic mesolimbic system plays a key role in the mechanisms of reinforcement and reward elicited by alcohol (ethanol) and other drugs of abuse. In addition, the dopaminergic activity of the nigrostriatal pathway has been proposed to determine brain sensitivity to ethanol, a process which could be associated to drug addiction. Besides dopamine, several neurotransmitters and neuromodulators are involved in ethanol reinforcement mechanisms, including gamma aminobutyric acid (GABA), glutamate, serotonin, acetylcholine and opioid peptides (enkephalins, endorphins and dynorphins). Ethanol and opioids share several pharmacological properties and exhibit similar behavioral effects in animals and humans. These and other studies suggest that the alcohol reinforcing properties are due, at least in part, to the ethanol-induced activation of endogenous opioidergic systems. This activation could in turn increase the hedonic value and the reinforcing effects of the drug. Thus, ethanol-induced changes in opioidergic transmission could contribute to alcohol intoxication and to the neuroadaptive responses produced by the long-lasting exposure to the drug. Opioidergic transmission may be altered by ethanol at different levels, including biosynthesis, release and inactivation of endogenous opioid peptides, as well as their binding to their receptors. Several studies suggest that mu and delta opioid receptors play a key role in ethanol reinforcement and dependence. Therefore, enkephalins and β -endorphin could physiologically mediate ethanol actions in the brain and play a major role in high drug use behavior. During the last few years, our research group has focused on the role of the endogenous opioid systems in these processes. Evidence obtained in our laboratory suggests that enkephalins and β -endorphin differentially and selectively participate in ethanol reinforcement and dependence.

Key Words: Alcohol (ethanol), reinforcement, dependence, endogenous opioid systems, enkephalins, β -endorphin, mesocorticolimbic system, nigrostriatal pathway.

RESUMEN

Evidencias bioquímicas y conductuales indican que el sistema dopaminérgico mesolímbico cumple un papel fundamental en los mecanismos de reforzamiento y recompensa del alcohol (etanol) y otras drogas de abuso. Se ha propuesto también que la actividad de la vía dopaminérgica nigroestriatal determina la sensibilidad cerebral a etanol, lo que parece estar directamente relacionado con los procesos de adicción a la droga. Además de la dopamina, varios neurotransmisores y neuromoduladores están implicados en los mecanismos de reforzamiento del etanol, entre ellos, el ácido gama-aminobutírico (GABA), el glutamato, la serotonina, la acetilcolina y los péptidos opioides (encefalinas, endorfinas y dinorfinas). El alcohol y los opioides comparten características farmacológicas y exhiben efectos similares sobre el comportamiento en animales y en el hombre. Éstos y otros estudios sugieren que las propiedades reforzadoras del etanol se deben, al menos parcialmente, a la activación de los sistemas endógenos de péptidos opioides, proceso que es inducido por el propio alcohol. Esta activación podría, a su vez, aumentar el valor hedónico y los efectos reforzadores de la droga. Los cambios inducidos por etanol sobre la transmisión de opioides podrían contribuir de manera importante a los procesos de intoxicación y a las respuestas neuronales adaptativas que produce el consumo prolongado de la droga. La transmisión opioidérgica puede ser afectada por etanol a distintos niveles, incluyendo la biosíntesis, liberación e inactivación de los opioides endógenos, así como la unión de éstos a sus receptores. Numerosas evidencias sugieren que los receptores opioides mu y delta desempeñan un papel fundamental en el reforzamiento y la dependencia al etanol. Así, las encefalinas y la β -endorfina actuarían como mediadores fisiológicos de las acciones del etanol en el cerebro, desempeñando un papel crucial en las conductas de alto consumo de la droga. En los últimos años, nuestro grupo se ha centrado en investigar el papel de los sistemas endógenos de péptidos opioides en estos procesos. Las evidencias obtenidas en nuestro laboratorio sugieren que las encefalinas y la β -endorfina participan en forma diferencial y selectiva en el reforzamiento y la dependencia al etanol.

Palabras clave: Alcohol (etanol), reforzamiento, dependencia, sistemas opioides endógenos, encefalinas, β -endorfina, sistema mesocorticolímbico, vía nigroestriatal.

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INTRODUCTION

Alcoholism is one of the main health problems worldwide and alcohol is one of the most extensively used drugs. In Mexico, alcoholism is associated to at least five out of the seven main causes of death throughout the country¹ and 25.4% of Mexicans aged 18-65 years old drink it in excess and/or have a dependence.² Due to the high prevalence of this disorder in Mexico, and since alcohol is a highly addictive drug, it is essential to know the action mechanism of alcohol in the brain – both in respect of the reinforcing properties on specific neural pathways and at the level of neuroadaptive changes caused by the long-lasting exposure to the drug in the brain. The knowledge of these mechanisms would significantly contribute to the design of new therapeutic strategies in the treatment of alcoholism.

During the last few years, our group has focused on researching the action mechanisms of alcohol on brain pathways that are part of the reinforcement and reward circuits of drugs of abuse (*i.e.*, mesocorticolimbic pathway). In particular, our interest is to investigate the participation of the endogenous opioid systems in the alcohol reinforcement and dependence mechanisms. Thus, we designed several experimental paradigms that have allowed us to study how the transmission of enkephalins and β -endorphin are modified in response to the acute and chronic ethanol exposure, particularly in brain areas of the mesocorticolimbic pathway. Also, we have correlated the neurochemical changes induced by ethanol with the behavioral effects caused by the drug. Here are some of the most important findings of our researches.

BACKGROUND

Alcohol is a depressing drug of the Central Nervous System (CNS) that affects a number of body functions, including temperature regulation, motor coordination and sleeping patterns. However, alcohol's effects in the CNS depend on the administered dose, especially at a behavioral level. Alcohol (ethanol) shows characteristic two-phase effects, both in animals and in humans. Low doses of the substance induce locomotive stimulation on rodents,³ as well as psychomotor activation and euphoria in humans,⁴ while high doses diminish locomotive activity and produces sedation.⁵ In the brain and in neuroendocrine tissues, ethanol modifies the activity of many neurotransmitters and neuromodulators, including dopamine (DA), serotonin, gamma aminobutyric acid (GABA), glutamate, acetylcholine, neuropeptide Y, corticotropin-releasing factor (CRF) and opioid peptides.⁶⁻⁹ These neural systems are differentially involved in the ethanol positive reinforcement and reward, as well as in the high drug-consumption behaviors.

Biochemical and pharmacologic evidences show that the dopaminergic (DAergic) mesolimbic system fulfills a significant role in the reinforcement and reward mechanisms of alcohol and other drugs of abuse.^{10,11} Several studies show that ethanol modulates DAergic transmission in the mesolimbic system and that activation of this pathway eventually leads to the development of an addictive behavior. Ethanol increases the triggering frequency of DAergic neurons in the ventral tegmental area (VTA)¹² and the release and metabolism of dopamine (DA) in the nucleus *accumbens* (NAcc) and the prefrontal cortex.^{13,14} The release of DA in the NAcc induced by ethanol is a critical event in the reinforcement and reward mechanisms of drugs of abuse.

Several neurotransmitters and neuromodulators, such as the opioid peptides (enkephalins, endorphins and dynorphins), modify the DAergic activity of the mesocorticolimbic system. Mu (μ) opioid receptor agonists, such as morphine, increase the triggering frequency of DAergic neurons in the VTA and stimulate the release and metabolism of DA in the NAcc and the prefrontal cortex, while delta (δ) opioid receptor agonists do not have any effect.¹⁶⁻¹⁸ These findings indicate that the activation of opioid receptors for endogenous opioid peptides is critical in the regulation of the DAergic transmission in the mesolimbic system. Furthermore, these studies indicate that the effects of opioid peptides in this pathway are similar to those caused by ethanol. In fact, it has been shown that opioids and ethanol share numerous pharmacological properties and have similar behavioral effects in animals and humans. For example, the administration of low doses of ethanol or opioids stimulates the locomotive activity through DAergic activation in the VTA, while high doses activate DAergic terminals in the NAcc.^{19,20} The activation of the mesocorticolimbic DAergic system by μ and δ agonists induces reinforcement, while the activation of the kappa (κ)-opioid receptor is related to dysphoria. These actions are mediated by an increase or reduction in the release of DA in the NAcc, respectively.²¹ Collectively, these studies suggest that dependence to opioids and alcohol is mediated by a common neurobiological mechanism involving the activation of reward DAergic circuits.¹¹

Participation of opioids peptides in alcohol reinforcement and dependence

Several biochemical and pharmacological evidences suggest that the alcohol positive reinforcement and the high drug-consumption behavior are mediated, at least partially, by a mechanism involving the activation of the endogenous opioid system induced by ethanol.^{6,21,22} Such activation would increase the hedonic value and the reinforcing ethanol's properties, which in turn would maintain high consumption behavior of the substance. Likewise, numerous evidences support the key role of the opioidergic sys-

tems in the mechanisms of ethanol reinforcement and the high drug-consumption behavior. The administration of low doses of μ opioid receptor agonists increases ethanol consumption in rats,²³⁻²⁵ while the administration of high doses diminishes it.^{23,26} The administration of non-selective antagonists (naloxone and naltrexone) of opioid receptors diminishes ethanol voluntary consumption in rodents and monkeys.²⁷⁻³¹ Selective antagonists of μ and δ opioid receptors also diminish ethanol consumption in different experimental paradigms.^{27,28,30,32-35} On the other hand, studies conducted in humans show that the administration of naltrexone in alcoholic patients reduces the compulsive behavior for drinking alcohol, as well as euphoria and the number of relapses.^{36,37} The use of knockout mice has allowed to confirm the participation of μ and δ receptors in the ethanol consumption behaviors.^{38,39} Collectively, these studies indicate that the activation of the β -endorphinergic and enkephalinergic systems, through the μ and δ receptors, is relevant in the ethanol reinforcement and in maintaining a high drug-consumption behavior.

The use of rodent strains and lines genetically selected for preferring alcohol and consume great amounts of the drug has contributed for the identification of the specific opioid systems and the neural substrates involved in the ethanol reinforcement. Additionally, the studies that use pharmacological manipulations of the opioidergic systems on ethanol consumption have assisted in investigating the key role of opioids in these behaviors. Nonetheless, studies

that have investigated these aspects in animals not genetically selected to prefer alcohol are scarce. Thus, our group has studied the effects of the acute and chronic ethanol administration over different events of the methionine-enkephalin (Met-enk) and β -endorphin transmission, using several experimental paradigms in Wistar rats. In particular, we studied ethanol effects on the mRNA expression of precursors that give rise to the Met-enk and to the β -endorphin (pro-endorphin [pro-enk] and pro-opiomelanocortin [POMC]), as well as on the content of the peptides in regions of the mesocorticolimbic system and of the nigrostriatal pathway. Furthermore, we investigated the effects of the acute and chronic ethanol administration over the binding of selective ligands of the μ and δ opioid receptors in those areas. Finally, we researched whether there is a correlation between the changes produced by ethanol on the transmission of these peptides and the behavioral effects induced by the drug.

Acute and chronic ethanol effects on the β -endorphinergic and enkephalinergic systems

Ethanol may induce important alterations on the biosynthesis, release and inactivation of opioids, as well as on the binding of endogenous opioid peptides to their receptors. Our initial studies were focused on studying the effects of the acute administration of a high ethanol on the binding

Table 1. Effect of an acute ethanol dose on a binding of [³H]-DAMGO to the mu opioid receptor in regions of the mesocorticolimbic system and of the nigrostriatal pathway of the rat

Region	Time after administration (h)			
	0.5	1	2	4
Ventral tegmental area	60.3 ± 7.3 ***	83.0 ± 8.2	107.6 ± 9.9	103.9 ± 6.1
Prefrontal cortex	99.9 ± 5.5	86.4 ± 3.8	138.1 ± 4.5 ***	108.1 ± 3.2
Nucleus <i>accumbens</i>				
• "core"	102.0 ± 4.2	83.5 ± 5.8	106.7 ± 5.5	91.1 ± 4.7
• "shell"	99.4 ± 3.3	80.7 ± 4.3 **	116.7 ± 3.8	92.2 ± 2.5
<i>Substantia nigra</i>				
• pars compacta	111.8 ± 5.6	95.5 ± 10.9	100.0 ± 11.1	99.0 ± 9.1
• pars reticulata	142.1 ± 10.2	33.4 ± 6.2 *	79.1 ± 17.9	105.4 ± 8.5
Caudate and putamen				
1. Anterior-medial				
• patches	98.9 ± 5.3	88.5 ± 3.7	103.0 ± 3.5	103.1 ± 3.0
• matrix	106.2 ± 4.5	96.3 ± 7.4	72.7 ± 9.0	94.8 ± 5.2
2. Medial-posterior				
• patches	89.3 ± 7.0	85.9 ± 7.2	100.2 ± 6.0	82.5 ± 6.0
• matrix	87.8 ± 8.3	123.2 ± 12.3	79.6 ± 24.2	142.8 ± 7.6
3. Posterior	84.4 ± 6.6	100.7 ± 7.5	94.3 ± 7.5	101.0 ± 6.2

Male Wistar rats received an acute ethanol dose (2.5g/kg) or water (control group) by intragastric pathway and were put down after 30min, 1, 2 or 4h of the administration. The binding of [³H]-DAMGO (8 nM) to the mu opioid receptor was studied by a quantitative autoradiography of receptors in coronal cuts of the brain. The data are expressed as a control percentage in each time studied, being the average ± EEM of 5 (control) or 6 (ethanol) animals. *** p<0.005; ** p<0.01; * p<0.05, versus controls in each time (two-way ANOVA).

Table 2. Effect of an acute ethanol dose on a binding of [³H]-DPDPE to the delta opioid receptor in regions of the mesocorticolimbic system and of the nigrostriatal pathway of the rat

Region	Time after administration (h)			
	0.5	1	2	4
Prefrontal cortex	73.4 ± 8.3	119.4 ± 13.9	377.4 ± 88.7***	100.5 ± 6.5
Nucleus <i>accumbens</i>				
• "core"	79.4 ± 10.2	121.0 ± 14.3	193.4 ± 34.6**	46.8 ± 10.2
• "shell"	90.0 ± 9.9	119.7 ± 13.4	197.1 ± 37.1*	45.9 ± 8.6
<i>Substantia nigra</i>				
• Pars reticulata	63.7 ± 14.2	266.6 ± 32.2***	85.2 ± 8.8	70.0 ± 17.3
Caudate and putamen				
1. Anterior-medial	83.5 ± 6.7	139.6 ± 9.2	244.6 ± 26.8***	93.9 ± 10.7
• lateral region	92.8 ± 6.9	120.0 ± 8.2	195.2 ± 17.0***	90.4 ± 7.7
2. Medial-posterior	116.4 ± 11.5	167.3 ± 11.1	221.4 ± 76.5**	103.0 ± 21.1
• lateral region	147.3 ± 11.1	137.3 ± 9.7	296.1 ± 65.5***	83.9 ± 15.1
3. Posterior	58.3 ± 7.5 *	133.1 ± 6.6	111.7 ± 15.8	121.3 ± 11.2

Male Wistar rats received an acute ethanol dose (2.5g/kg) or water (control group) by intragastric pathway and were put down after 30min, 1, 2 or 4h of the administration. The binding of [³H]-DPDPE (8nM) to the delta opioid receptor was studied by a quantitative autoradiography of receptors in coronal cuts of the brain. The expression levels of the delta receptor in the ventral tegmental area and the *substantia nigra pars compacta* are very low and could not be quantified in this study. The data are expressed as a control percentage in each time studied, being the average ± EEM of 5 (control; ethanol 30min and 2h) or 6 (ethanol 1 and 4h) animals. *** p<0.0001; ** p<0.01; * p<0.05, versus controls in each time (two-way ANOVA).

of selective ligands of the μ and δ receptors in brain areas associated with the reinforcement and reward circuits of drugs of abuse. We also studied the acute ethanol effects on the binding of these ligands in areas of the nigrostriatal pathway, since it has been proposed that the DAergic activity of this pathway determines the brain sensitivity to ethanol.⁴⁰ We found that the administration of a 2.5 g/kg ethanol dose in Wistar rats alters the binding of selective ligands of the μ ([³H] [D-Ala²,MePhe⁴,Gly-ol⁵]-enkephalin) ([³H]-DAMGO) and δ ([³H] [D-Pen²,D-Pen⁵]-enkephalin) ([³H]-DPDPE) receptors, differentially, in discreet areas of the rat brain, as well as with different kinetic patterns (Tables 1 and 2). The acute ethanol administration increased the binding of [³H]-DPDPE to the δ receptor in the prefrontal cortex and the NAcc (*core* and *shell*)⁴¹ (Table 2), as well as the binding of [³H]-DAMGO to the μ receptor in the prefrontal cortex⁴² (Table 1). The same treatment diminished the binding of [³H]-DAMGO in the VTA and the *shell* of the NAcc⁴² (Table 1). In the nigrostriatal pathway, we also found important differences regarding the acute ethanol effects and the response kinetics (Tables 1 and 2). The administration of the same ethanol dose reduced the binding of [³H]-DAMGO (Table 1), but increased the binding of [³H]-DPDPE (Table 2) in the *substantia nigra, pars reticulata* (SNr).^{41,43} This treatment also increased the binding of [³H]-DPDPE in the caudate and putamen (CP),⁴¹ but it did not modified the binding of [³H]-DAMGO in this region of the brain.⁴³

The studies described suggest that the ethanol reinforcing properties could be partially measured by positive and negative regulatory mechanisms of the μ and δ receptors

in different areas of the mesocorticolimbic and nigrostriatal pathways (for reviews, refer to Méndez and Morales-Mulia, 2008a,b).^{44,45} According to this proposal, the μ receptors in the VTA would develop a key role in the regulation of the DAergic activity of the mesocorticolimbic system in response to ethanol, while the μ and δ receptors would play a predominant role in the NAcc and the prefrontal cortex. In the nigrostriatal pathway, both types of receptors would have an important role at the level of the *substantia nigra* (SN), but only δ receptors seem to be involved in the DAergic modulation in the CP. These results indicate that the modulation of the DAergic transmission in these pathways –through the activation of μ and δ receptors– take place by different mechanisms, and that the β -endorphinergic and enkephalinergic transmission performs a key role in the ethanol reinforcement.

The next stage of our research was studying the acute ethanol effects on the Met-enk and β -endorphin expression in brain areas of the reinforcement and reward circuits. The administration of a high ethanol dose (2.5g/kg) diminished the expression of the mRNA of pro-enk in the VTA and the SN (*pars compacta* [SNc] and SNr) of Wistar rats, and increased it in the prefrontal cortex.^{46,47} Interestingly, this treatment causes a prolonged and sustained increase in the levels of the mRNA of pro-enk in the NAcc, both in the *core* and in the *shell*,⁴⁶ as well as in different zones of the CP.⁴⁷ The ethanol administration also produces increases in the expression of this mRNA in other brain areas –such as the hypothalamic paraventricular nucleus–, while in others –as the dentate gyrus and the CA1, CA2 and CA3 regions of the hippocampus– ethanol induces two-phase effects.⁴⁸ On the other hand, the administration of a 2.5g/kg ethanol dose reduced the contents of

Met-enk in the NAcc and the CP, but had no effect in the prefrontal cortex.⁴⁹ Low or intermediate ethanol doses (0.25, 0.5 and 1g/kg) did not modify the contents of the peptide in these brain areas.⁴⁹ In contrast with the effects produced by different ethanol doses on the contents of Met-enk in the said regions, other studies of our laboratory revealed that ethanol, in intermediate and high doses, stimulates the release of Met-enk in the NAcc of the rat.⁴⁹ The maximum effect was detected with a 1g/kg ethanol dose and the response kinetics to the ethanol administration was presented differentially. The set of these studies indicate that the enkephalinergic system is one of the important targets of ethanol in the brain and the Met-enk release induced by ethanol is one of the key events in such actions (for reviews, refer to Méndez and Morales-Mulia 2008b,c).^{45,50} These studies suggest, in addition, that the enkephalins perform a crucial role in the ethanol reinforcement. The modulation of the DAergic transmission for opioids in the mesocorticolimbic system is, undoubtedly, an important part of this process.

In parallel to the described studies, we also investigated the effects of the administration of a single ethanol dose on the β -endorphinergic transmission. However, the administration of a 2.5g/kg ethanol dose did not modify the contents of β -endorphin in regions such as the VTA, the SN, the NAcc or the prefrontal cortex.⁵¹ In contrast, the same treatment diminished the contents of the peptide in the hypothalamus. These results suggest that the β -endorphinergic system participates selectively in the ethanol action mechanisms that are more associated to the neuroendocrine effects of the drug. Studies now conducted in our laboratory shall confirm if this is the case. In particular, our interest is focused on assessing the changes induced by the ethanol administration on the expression of the mRNA of POMC in different areas of the brain. In addition to the hypothalamus, brain regions of the reinforcement circuits, like the VTA, the NAcc or the prefrontal cortex, contain very low levels of this mRNA.⁵² Currently, we assess whether the exposure to a high ethanol dose modifies or not the expression of this mRNA in the rat. Other studies of our group, in conjunction with Dr. Bérrod's study, show that the acute administration induces the expression of Fos in GABAergic and non-GABAergic neurons in the midbrain of the rat.⁵³

In order to investigate if the neurochemical effects of ethanol on the opioidergic transmission correlate with the behavioral actions of the drug, we carried out a series of experiments in which we studied the effect of different alcohol doses on the motor activity in the rat. We observed that the 1g/kg ethanol doses increase the horizontal moving and the stereotypes, while high doses of the substance (2.5g/kg) diminish these parameters and have a sedative effect.^{54,55} Thus, the alterations induced by high alcohol doses on the transmission of opioids in the brain, previously described, could be related to the sedative effects of the drug. Likewise,

several factors may significantly modify the motor response to the alcohol's administration. In our laboratory we have found that factors such as the circadian cycle, the isolation or the exposure of animals to novel environments have very important effects on the locomotive behavior of the rat.⁵⁴⁻⁵⁶ These and other factors appear to be decisive in the behaviors of high consumption of alcohol and other drugs of abuse.

Furthermore, the analysis of the studies of alcohol chronic exposure revealed interesting results. The chronic treatment with ethanol (10% v/v, four weeks) did not modify the binding of [³H]-DPDPE in the VTA, the prefrontal cortex, the NAcc and the anterior-medial region of the CP.⁵⁷ Nonetheless, we observed a decreasing trend in the binding of the ligand in the SNc and the medial-posterior region of the CP, a result that is currently being confirmed in our laboratory. The binding of [³H]-DAMGO to the μ receptor in the VTA, the prefrontal cortex or the NAcc was neither modified by prolonged ethanol exposure.⁵¹ Although these studies show that chronic ethanol exposure for four weeks does not modify the binding of the ligands used, whether this type of treatment induces changes in the functionality of μ and δ receptors in the studied regions remains to be investigated.

In contrast with the data obtained in the acute treatment, chronic ethanol exposure selectively increased the contents of Met-enk in the VTA and the prefrontal cortex (Table 3), but it did not modify the peptide concentration in the NAcc, the CP, the SN, the hippocampus and the amygdala.⁵⁷ The same treatment did not modify the contents of β -endorphin in none of these areas⁵¹ (Table 3). These results suggest that the mesocortical enkephalins take place in the neuroadaptive changes that occur during prolonged ethanol exposure.

With the purpose of comparing the chronic effects of a drug of abuse (alcohol) with those effects caused by natural reinforcers such as sugars, we also investigated the effect of chronic sucrose exposure within the same experimental paradigm. Sucrose exposure during four weeks increased the contents of Met-enk in the NAcc and the hypothalamus⁵⁷ and of β -endorphin in the NAcc and the SN⁵¹ (Table 3). The binding of the [³H]-DAMGO in the VTA, the prefrontal cortex and the NAcc was not affected by the sucrose. In contrast, the sugar augmented the binding of [³H]-DPDPE in the prefrontal cortex and in the anterior-medial region of the CP. Furthermore, we observed a growing trend in the binding of [³H]-DPDPE in the NAcc (*core* and *shell*) and the SNr in animals chronically treated with sucrose, an effect that is being confirmed in our laboratory. Such results suggest that the enkephalins and the β -endorphin present in the NAcc, the SN and the hypothalamus participate in the mechanisms that regulate the palatability of substances.

The set of these studies suggests that the prolonged alcohol and/or sucrose exposure induces selective changes

Table 3. Effect of chronic ethanol and sucrose exposure on the contents of methionine-enkephalin and β -endorphin in regions of the brain of the rat

Region	Ethanol		Sucrose	
	Met-enk	β -endorphin	Met-enk	β -endorphin
Ventral tegmental area	↑ 25.3% **	—	—	—
Prefrontal cortex	↑ 245.0% ***	—	—	—
Nucleus accumbens	—	—	↑ 61.1% *	↑ 100.0% *
Substantia nigra	—	—	—	↑ 65.0% *
Caudate-putamen				
• anterior-medial	—	nd	—	nd
• medial-posterior	—	nd	—	nd
Hypothalamus	—	—	↑ 55.8% *	—

Male Wistar rats were chronically treated with ethanol (10% v/v), sucrose (isocaloric control) or water during 4 weeks. Food and liquid intake was matched with the ethanol group and also an *ad libitum* group was included with free access to food and water. The contents of the peptides were determined by radioimmunoassay. The data are expressed as the percentage of average change with regard to the *ad libitum* group (n=8-10 per group). The changes marked with an arrow were significant as for the *ad libitum* group (one-way ANOVA). *** p<0.001; ** p<0.01; * p<0.05; nd=not determined; —=without effect.

in enkephalinergic and/or β -endorphinergic neurons in discreet areas of the rat brain. The changes observed in the enkephalinergic system could be related to the alcohol and/or sucrose reinforcement mechanisms, while those found in the β -endorphinergic system seem to be associated with the sucrose reinforcement.

CONCLUSIONS

The set of studies conducted in our laboratory suggests that the biosynthesis and release of opioid peptides (Met-enk and β -endorphin), as well as the activation of δ and μ receptors stand for key events in the opioidergic transmission in the mesocorticolimbic and nigrostriatal pathways subject to regulation for the acute and chronic alcohol exposure. The ethanol effects, besides depending on the dose and the duration of the drug exposure, are of a specific target region. The changes detected in the enkephalinergic and β -endorphinergic systems in animals chronically treated with ethanol probably are part of the neuroadaptations progressively established in the brain during the prolonged drug exposure. Finally, our results suggest that these peptidergic systems take part in the alcohol and/or sucrose reinforcement mechanisms. Particularly, the enkephalinergic system seems to be involved in the alcohol and sucrose reinforcement, while the β -endorphinergic system appears to be more linked to the sucrose reinforcement. It remains to be seen whether during alcohol and/or sucrose deprivation (abstinence) specific changes occur in these peptidergic systems as for the studied regions, as well as the relevance of same in the mechanisms of dependence to these substances.

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