Action of mescaline (mesc) alone or in combination with other agents affecting catecholamine action and metabolism

Treatment	Day 1 SL	Day 2 SL	Day 3 SL	MAO (μmoles/h)	Monoamines (μg)		
					DA	NE	5HT
Controls	— 4.0 ± 1.9	— 8.0 ± 4.7	— 8.3 ± 4.1	5.8	2.15 ± 0.07	0.46 ± 0.02	0.35 ± 0.05
Mesc (10^{-5} *M*)	— 5.8 ± 0.4	2.2 ± 1.0	19.6 ± 10.0	5.4	1.28 ± 0.01	0.44 ± 0.01	0.31 ± 0.01
Controls	— 3.0	— 4.0	— 2.0		1.50 ± 0.02	0.47 ± 0.01	0.53 ± 0.01
Mesc (10^{-4} *M*, 3 h/day)	— 10.0	— 14.0	— 21.0		1.91 ± 0.02	0.60 ± 0.01	0.52 ± 0.01
L-dopa (10^{-4} *M*)	— 9.0	— 12.6	— 17.0	5.3	2.17	0.48	
Mesc (10^{-5} *M* + dopa)	— 10.0	— 11.2	— 10.8	5.8	1.96	0.43	
Deprenyl (10^{-6} *M*)	— 31.4	— 34.9	— 35.6	0.1	2.75		
Mesc (10^{-6} *M* + depren.)	— 4.0	— 9.6	— 13.0	0.1	2.87		
Fluphenazine (10^{-6} *M*)	— 3.0	5.0	22.0	7.9	1.94	0.45	
Fluph. + dopa (10^{-4} *M*)	— 3.4	— 8.0	— 14.4	7.1			
Apomorphine (10^{-6} *M*)	— 14.2	— 24.8	— 21.6	3.9	2.47	0.52	

The MAO activity was measured with 10^{-3} *M* tyramine; the hydrogen peroxide produced in this reaction converted homovanillic acid into a fluorescent compound in the presence of horse radish peroxidase[11]. The cerebral amines were determined after 3 and 72 h of exposure of the fish to the drugs; after extraction they were converted to fluorescent compounds with *o*-phthaldialdehyde (5HT) or iodine (DA, NE)[12]. MAO activities and amine levels were calculated for 1 g of fresh brain. The standard deviations were computed on the basis of data obtained for 18–36 fish, except monoamine determinations (6 fish). All groups, with the exeption of the second one, were exposed to the drugs for 24 h a day. Abbreviations: SL, slope of the linear relationships as defined in the text; DA, dopamine; NE, norepinephrine; 5HT, serotonin.

sible to differentiate 'short' and 'long' mescaline exposure: when we exposed the fish to a much higher concentration of mescaline for only 3 h prior to the training period, the slopes of the linear functions were significantly more negative than those obtained from controls ($p < 0.005$) and almost identical with those resulting from L-dopa application; no decrease in dopamine levels occurred. Thus, the phenomena recorded for short and long exposures to mescaline are dramatically different.

Our conclusion that dopamine – either derived from administered L-dopa or released by mescaline – is capable of increasing learning rate does not stand alone, as the following examples show: in rats, L-dopa improved learning in conditioned avoidance tests[6] while in mice the same drug reversed impaired learning due to the destruction of the dopaminergic nigrostriatal projection[7]. In a long series of studies, initiated by Arbit, et al.[8] and reviewed and extended by MURPHY, L-dopa was found to enhance learning in parkinsonian and non-parkinsonian patients.

To illustrate the second, mescaline-induced dopamine deficient state, few unambiguous data can be found in the literature, apparently because the distinction between the 'acute' and 'chronic' states has not been made before. Muscle rigidity and tremor observed in dogs and monkeys after the administration of very large doses of mescaline may suggest a dopamine loss[9].

Many times, similarities between mescaline-produced syndromes and certain schizophrenic reactions have been noted[2]. Since hyperactivity of a dopaminergic system is thought to be involved in the pathogenesis of (paranoid) schizophrenia (as discussed by SNYDER et al.[10]), abnormally high cerebral dopamine levels may be the common denominator of acute mescaline action and some schizophrenic symptoms. This concept throws some light on many earlier observations[2], e.g. on the reversal of mescaline responses by phenothiazines.

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Effects of Morphine Administration on Cerebellar Guanosine 3′,5′-Monophosphate

W. E. ASKEW and K. D. CHARALAMPOUS

Department of Psychiatry, Baylor College of Medicine, 1200 Moursund Avenue, Texas Medical Center, Houston (Texas 77030, USA), 9 February 1976.

Summary. An increase in mouse cerebellar C-GMP levels during acute morphine treatment was observed, which was possibly related to the decrease in C-GMP phosphodiesterase levels also observed in acute treatment. Chronic treatment lowered C-GMP levels as did abrupt withdrawal without naloxone.

Although a role for adenosine 3′,5′-monophosphate in morphine dependence has been tentatively described[1,2] the involvement of guanosine 3′,5′-monophosphate (C-GMP) is less well understood. BONNET[3] has reported significant reduction in C-GMP levels in rat caudate, substantia nigra, hypothalamus, and thalamus with acute morphine injections in both normal and morphine pelleted animals. However, TRABER et al.[4] have found that acute opiate exposure of neuroblastoma × glioma hybrid cells elevated intracellular C-GMP levels. To clarify the

Cyclic-GMP levels, guanylate cyclase, and C-GMP phosphodiesterase activities in mouse cerebellum as effected by acute (20 mg/kg and 40 mg/kg) morphine administration, by chronic administration, and by abrupt withdrawal with naloxone (0.05 mg/kg) and without naloxone

Treatment	C-GMP level	Guanylate cyclase	C-GMP phosphodiesterase
Acute (20 mg/kg)	158 (± 18) [a]	97 (± 8)	57 (± 12) [b]
Acute (40 mg/kg)	157 (± 16) [a]		
Chronic (24 h)	58 (± 15) [a]	83 (± 7) [b]	89 (± 14)
Chronic (48 h)	81 (± 20)	98 (± 11)	95 (± 19)
Chronic (72 h)	114 (± 13)	81 (± 13) [a]	84 (± 15)
Abrupt withdrawal (78 h)			
Without naloxone	70 (± 14) [a]	112 (± 15)	96 (± 12)
With naloxone	110 (± 22)	123 (± 17)	

Control C-GMP levels were 1. Acute: 2.77 pmoles/mg protein; 2. Chronic: 1.22 pmoles/mg protein; 3. Withdrawal: 2.26 pmoles/mg. Control guanylate cyclase activity was 0.49 nmoles/mg/min; control C-GMP phosphodiesterase activity was 1.52 nmoles/mg/min. Values are expressed here as a percentage of the matched control ± SD.
[a] $p < 0.05$; [b] $p < 0.01$. $n = 5$. The Student's t-test was used for comparison of experimental and control values.

effect of morphine administration on C-GMP levels, we have studied the effect of acute and chronic administration, as well as natural and naloxone precipitated withdrawal, on C-GMP levels in the mouse cerebellum where C-GMP levels are higher than in other brain areas[5].

Methods. For the acute studies, male albino Tex: ICR Swiss mice, 18–20 g, were injected i.p. with 20 mg/kg and 40 mg/kg morphine sulfate in a saline vehicle calculated as the free base and sacrificed after 30 min. Chronic animals were treated via morphine pellet implantation[6]. Each morphine pellet contained 75 mg of morphine base, 75 mg of microcrystalline cellulose, 0.75 mg of fumed silicone dioxide, and 1.5 mg of magnesium stearate. Placebo pellets were similarly prepared without morphine base. Mice treated with the pellet implantation technique are considered to be dependent after 72 h of exposure[6]. The time intervals studied during chronic treatment, or dependence development, were 24, 48, and 72 h after implantation. Withdrawal was initiated by removal of the morphine pellet 72 h after implantation with the time of pellet removal being considered as the zero time into withdrawal. For naloxone induced withdrawal, naloxone was administered 5 h and 50 min after pellet removal. Withdrawal symptomatology was then evaluated by the platform jumping technique[6]. Dependence was demonstrated by the jumping response in 92.3% of the mice tested, as effected by 0.05 mg/kg naloxone, which corresponds well to the 92% value reported with Simonsen ICR mice[7]. As reported elsewhere, mice undergoing abrupt withdrawal without naloxone did not demonstrate a consistent withdrawal symptomatology when tested via the platform jumping technique[6].

All animals were sacrificed at 6 h into withdrawal. Animals utilized in the C-GMP studies were sacrificed in a microwave oven. Cerebellar tissue was homogenized in absolute ethanol followed by centrifugation at $8{,}000 \times g$. An aliquot was evaquated to dryness under dry nitrogen, and C-GMP was assayed as described by STEINER et al.[8]. Protein levels were estimated according to the method of LOWRY et al.[9]. For the determinations of guanylate cyclase and C-GMP phosphodiesterase activities, animals were sacrificed by decapitation. Guanylate cyclase activities were determined as described by THOMPSON et al.[10], and C-GMP phosphodiesterase activities were measured according to RUSSELL et al.[11], and THOMPSON and APPLEMAN[12].

Results and discussion. The data obtained with the morphine treatments is presented in the Table. Acute control C-GMP values were 2.77 pM/mg protein or 0.27 pM/mg of wet weight. This corresponds well to a literature value of 0.28 pM/mg wet weight, using a similar type of microwave sacrifice[13]. The surgical trauma effected a decrease in cerebellar C-GMP values independent of drug treatment. Control guanylate cyclase and C-GMP phosphodiesterase activities were not appreciably affected by the course of treatment.

Acute treatment significantly increased C-GMP levels whereas chronic treatment, at 24 h, and abrupt withdrawal at 6 h into withdrawal, evidenced a significant decrease. Guanylate cyclase activities were significantly lower than control values at 24 h and 72 h during chronic treatment. Cyclic-GMP phosphodiesterase activity was significantly decreased by acute treatment.

Guanylate cyclase activity is dependent upon manganese and calcium[14]. Since acute morphine treatment produces a general decrease in calcium levels[15], the decrease

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in cerebellar C-GMP observed at 24 h during dependence development may reflect an effect of morphine on cerebellar calcium and hence on guanylate cyclase activities. The increase in cerebellar C-GMP following acute treatment is apparently the result of the observed phosphodiesterase inhibition and appears not to be dose related at least at the dosages examined.

No significant difference in cerebellar C-GMP was observed between control and drug animals at 6 h during naloxone precipitated abrupt withdrawal, however, drug values were less than control values in abrupt withdrawal without naloxone. These results may reflect naloxone stabilization of calcium levels as reported by Ross et al.[15] with the C-GMP decrease during natural withdrawal reflecting an acute calcium loss.

Our results then confirm the report of TRABER et al.[4] in that an increase in C-GMP was observed with acute treatment. Additionally, we observed the decrease in neural C-GMP in morphine pelleted rats as previously reported by BONNET[3]. A decrease in cerebellar C-GMP 24 h after pellet implantation was also observed.

Although the data is insufficient to describe a role for C-GMP in the development of morphine dependence and withdrawal, it seems apparent that the etiology of morphine dependence may be related to the level of this cyclic nucleotide in the cerebellum.

Die Wirkung einer Leberschädigung durch Thioacetamid auf die mikrosomale Aromatisierung von Testosteron bei der Ratte

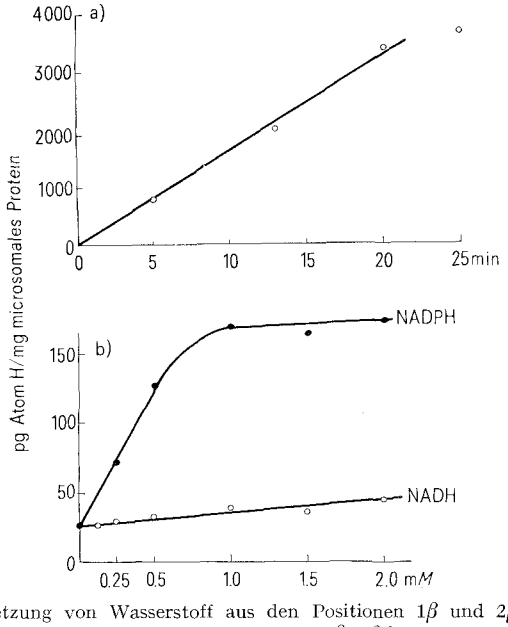
Effect of Liver Damage by Thioacetamide on Microsomal Aromatization of Testosterone in Rats

V. LÓPEZ DEL PINO und H. M. BOLT

Institut für Toxikologie der Universität Tübingen, Wilhelmstrasse 56, D-7400 Tübingen (Bundesrepublik Deutschland), 20. Mai 1976.

Summary. Rat liver microsomes, NADPH-regenerating system, and 1β, 2β-^{3}H-testosterone have been incubated in vitro. The loss of tritium from the steroid, associated with aromatization of testosterone, was linear with time for 20 min and required NADPH. Pre-treatment of the rats with thioacetamide raised the liberation of tritium from 1β, 2β-^{3}H-testosterone. The results suggest that liver damage by thioacetamide in rats may give rise to increased aromatization of testosterone.

Die Thioacetamid-vergiftete Ratte spiegelt als tierexperimentelles Modell Veränderungen des Östrogenabbaus wieder, die bei der menschlichen Leberzirrhose auftreten und hier zu klinischen Symptomen wie Gynäkomastie oder Hodenatrophie Anlass geben können[1]. Bisher galt dabei das Augenmerk in erster Linie Störungen des Östrogenabbaus, besonders der eingeschränkten Möglichkeit zur mikrosomalen 2-Hydroxylierung in der Leber[2,3]. Andererseits wurde postuliert[4], dass bei der Leberzirrhose des Menschen darüber hinaus eine periphere Bildung von Östrogenen aus androgenen Vorläufern in vermehrtem Masse stattfinde. Dies gab uns Anlass, nachzuprüfen, ob auch bei der Thioacetamidvergiftung der Ratte in vermehrtem Masse Androgene aromatisiert werden. Unsere Untersuchungen wurden in vitro durchgeführt mit Lebermikrosomen von Ratten, die akut oder chronisch mit Thioacetamid behandelt waren. Um einen Einblick in die insgesamt aus Testosteron gebildete Menge aromatischer Folgeprodukte zu erhalten, wurde als Substrat 1β,2β-^{3}H-Testosteron verwendet. Bei der Aromatisierung des A-Ringes zu Östrogenen wird bevorzugt der an 1β und 2β gebundene Wasserstoff eliminiert[5] und kann dann im Inkubationsmedium als HTO gemessen werden. Da für unsere Fragestellung lediglich der relative Effekt einer Thioacetamidbehandlung in bezug auf unbehandelte Kontrolltiere interessierte, konnte dieses Vorgehen gewählt werden.



Freisetzung von Wasserstoff aus den Positionen 1β und 2β von Testosteron in vitro bei Inkubation von 1β, 2β-^{3}H-Testosteron mit Rattenlebermikrosomen (Kontrollratten) und NADPH-regenerierendem System. a) Zeitabhängigkeit der Reaktion. b) Abhängigkeit vom Kofaktor, wenn statt des NADPH-regenerierenden Systems verschiedene Konzentrationen an NADH (○—○—○) bzw. NADPH (●—●—●) eingesetzt werden.

Experimenteller Teil. 1β,2β-^{3}H-Testosteron wurde von der Firma New England Nuclear Inc., Boston, Mass., bezogen.

Die akute Vorbehandlung von Ratten mit Thioacetamid erfolgte durch p.o. Verabreichung der Substanz in wässriger Lösung. Die Dosierungen betrugen 100 mg/kg, 200 mg/kg und 300 mg/kg. 24 h nach der Behandlung wurden die Ratten getötet. Die Präparation von Leber-

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