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Activity of lysosomal enzymes in the liver and kidneys of mice after morphine administration

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Used were ninety Swiss mice males divided into 9 groups (n=10). Three control groups [I, II, III] were injected with 0.9% NaCl solution, while six experimental groups (A, B, C, D, E, F) with the morphine hydrochloride. Mice from groups A, C and E were injected with a dose of 20 and those from B, D and F with a dose of 30 mg morphine per kg body weight. In each group, both solutions were administered intramuscularly once a day from 9:00 to10:00 a.m. for 4, 10 and 14 days.
In the lysosomal fraction of the liver and kidney the activities of cathepsin D, cathepsin L, alanine aminopeptidase, leucine aminopeptidase, lysosomal lipase, and β-glucosidase were estimated. Morphine led to the increased activity of all examined enzymes except lysosomal lipase, the activity of which dropped in both organs examined.

KEY WORDS: kidney / liver / lysosomal hydrolases / mice / morphine

As far as fight against pain is concerned, opioids including morphine are one of the most important groups of pharmacological agents **[**Sjögren i Żylicz 2004]. It doesn't mean that they are effective in attenuating and annihilating all types of pain. However, they are still irreplaceable [Kochanowski *et al*. 2005]. Mechanism of morphine's analgesic action has not been fully recognized yet. It influences neither

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sensory nerve endings nor a course of pain impulse in these sensory nerves. Pain killing action depends principally upon functioning of a central nervous system and consists of inhibition of reaction, mainly of limbic system and thalamus [Chamoles *et al*. 2002]. Nevertheless, it is not known which biochemical phenomenon determines its analgesic properties.

This problem seems to be interesting, especially with regard to specific role of lysosomes and lysosomal enzymes in the cells of mammals [Lendeckel *et al*. 1999, Uchiyama 2001, Steet *et al*. 2005]. Their role, with reference to adaptive processes elicited by an action of opiates, particularly morphine, remains unexplained. The aim of this experiment was to identify an impact of two doses of morphine and their administration time upon activity of the selected lysosomal hydrolases in the mouse liver and kidney. Such information we had not find in the available literature.

Material and methods

The study was conducted on 90 Swiss male mice, at the age 8-9 weeks, with mean body weight 23.0±1.3 g, randomly selected from more than 1000 animals maintained on a mouse farm at the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences, Jastrzębiec. The mice were kept under the standard breeding conditions, in the air conditioned room with 50%-60% relative humidity, 21°-22°C and 12-hour light cycle, fed on granulated commercial feed with standard 16% protein content and with constant access to water.

Arranged were 9 groups of 10 mice – three control (I, II III) and six experimental (A, B, C, D, E, F). Once a day, between 9:00 and 10:00 a.m., over a period of 4, 10 and 14 days, mice from experimental groups were injected intramuscularly with morphine hydrochloride (Morphinum hydrochloricum, Polfa, Warsaw). Mice from groups A, C and E were given 20 mg and those from B, D and F – 30 mg morphine per kg body weight (BW) in 250 µl 0.9% NaCl per mouse. These values are analogous to those applied in human medicine per 24 h. Mice from control groups (I, II, III) were injected with 250 µl of 0,9% NaCl solution per mouse over 4, 10 and 14 days.

Four hours after the last injection of morphine hydrochloride or 0,9% solution of NaCl mice were decapitated and liver and kidney samples immediately homogenized according to Beaufay [1972] method. The activity of cathepsins D and L (Cath.D, EC 3.4.23.5; Cath.L, EC 3.4.22.15) were determined with Langner *et al*. [1973] method, alanine aminopeptidase (AlaAP, EC 3.4.11.2) and leucine aminopeptidase (LeuAP, EC 3.4.11.1) with McDonald and Barrett [1986] method, lysosomal lipase (LL, EC 3.1.1.13) with Barrett and Heath [1977] method, and β-glucosidase (EC 3.2.1.21) according to Barrett [1972] method were determined in the supernatants of liver and kidneys obtained. Moreover, the supernatant protein level was determined with Kirschke and Wiederanders [1984]. Activity of enzymes was expressed in nmol/mg protein/hour.

The statistical analysis has been based on the programme SAS/STAT [1999-2001, User's Guide, SAS Institute Inc., Cary, NC, USA] and Original (version 5.0, Microcal Software Inc. Northampton, USA).

This experiment was approved by Local Committee for Ethics in the Animal Experimentation of the Institute of Genetics and Animal Breeding, Jastrzębiec (Permission No. 18/99).

Results and discussion

There are numerous studies concerning lysosomes and their enzymes [Witek *et al*. 1999, Zatta *et al* 2000, Bandyopadhyay and Cuervo 2008] Results seem to suggest that lysosomes play a significant role in cell's regulatory processes and that changes in activity of lysosomal hydrolases are important factors maintaining the specific cell interior adequate for its need for homeostatic system, enabling an adaptation to changing environmental conditions [Fokens *et al.* 1998, Witek and Kołątaj 1999].

Data from Tables 1-4 indicate that morphine administration in the doses of 20 and 30 mg/kg BW caused highly significant or significant increase in Cath. D and L

Table 1. Means and standard deviations (SD) for lysosomal enzymes activities (nmol/mg of protein/h) in the liver of mice injected over 4 days with morphine hydrochloride

Enzyme	Control groups		Experimental groups					
			20 mg morphine daily per kg body weight			30 mg morphine daily per kg body weight		
	mean	SD	mean	SD	per cent of control	mean	SD	per cent of control
Cath. D and L	7.67	1.58	9.82*	3.73	128	10.17**	2.25	133
AlaAP	8.38	1.54	8.22	2.47	98	9.76	1.23	116
LeuAP	8.78	2.24	10.37	3.02	118	10.77*	2.15	123
LL	7.20	2.60	6.53	2.08	91	5.97	2.16	83
β-Glu	1.56	0.27	1.65	0.56	106	2.05**	0.70	132

*, **, ***Statistically different from control at P≤0.05, P≤0.01 and P≤0.001, respectively.

activity both in the liver and in kidneys of mice, after every single examined period – 4, 10 and 14 days of injections (Tab. 1-6). Singhal *et al*. [1995] studied an influence of the morphine on Cath. D and L activity in isolated glomerular cells and demonstrated the significant activity increase. It may indicate an acceleration of proteolysis caused by a necessity of degradation exo- and endogenous proteins. It is now proved that morphine contributes towards increasing the level of glycosaminoglycans and probably also towards increasing an activity of degrading enzymes including Cath. D. Glycosaminoglycans (GAGs) – the main element of standard substance of connective tissue – are degraded in hepatocyte lysosomes and their metabolites are excreted

with urine [Głowacki *et al*. 1995, Kowalewski *et al*. 2008]. Significant increase in catheptic activity after administration of morphine hydrochloride probably associates with, caused in that way, significant growth of total content of GAGs in liver and kidneys and also accelerates their metabolism [Sandya and Sudhakaran 2007, *Ma et al*. 2008].

Morphine administration in the doses of 30 mg/kg BW over 4 days (Tab. 2) and 20 mg/kg BW over 14 days (Tab. 6) raised AlaAP activity in kidneys (P≤0.5). When injected over a period of 10 days, both doses of morphine (Tab. 3 and 4) increased the ALaAP activity in liver and kidneys. Increase of AlaAP activity let us make a suggestion that protein degradation was accelerated in both examined organs. This assumption is in accordance with Ostrowska [1993], who was able to demonstrate an active role of alanine aminopeptidase in regulating the protein degradation. Thus, morphine injected in doses applied in the current study activated indirectly – by increasing the activity of AlaAP – the rate of proteolysis in both organs examined [Larrinaga *et al*. 2005].

Table 2. Means and standard deviations (SD) for lysosomal enzymes activities (nmol/mg of protein/h) in the kidneys of mice injected over 4 days with morphine hydrochloride

Enzyme	Control groups		Experimental groups					
			20 mg morphine daily per kg body weight			30 mg morphine daily per kg body weight		
	mean	SD	mean	SD	per cent of control	mean	SD	per cent of control
Cath. D and L	6.73	1.19	8.49*	1.68	126	8.60*	2.00	128
AlaAP	7.64	1.45	7.83*	1.17	102	9.16*	1.51	120
LeuAP	7.74	0.87	9.28*	0.76	120	9.45*	1.06	122
LL	6.93	0.81	6.44	0.62	93	5.54*	0.65	80
β-Glu	1.11	0.12	1.44*	0.20	130	1.66***	0.23	150

*, ***Statistically different from control at P≤0.05 and P≤0.001, respectively.

Activity of LeuAP increased significantly – in the liver after day 4 of morphine injections with 30 mg/kg BW (Tab. 1), in kidneys after morphine in doses 20 and 30 mg/kg BW for 4 days (Tab. 2, P≤0.5), and in kidneys and liver after 10 days of administration of morphine (Tab. 3 and 4). Observed increase in the LeuAP activity was related, similarly as in AlaAP, to acceleration of protein degradation in lysosomes [Kochanowski *et al*. 2005]. It may suggest that morphine indirectly accelerates hydrolytic decomposition in both examined organs [Irazusta *et al*. 2003].

As shown in Table 2, activity of LL decreased (P≤0.5) – in kidneys after 4 days of injections with 30 mg/kg BW, and in liver and kidneys after 10 days of injections with both 30 and 20 mg morphine/kg BW (Tab. 3 and 4). LL seems to play the main role in maintaining homeostatic concentration of cholesterol [Sadurska and Skalska-Hilgier 2001] and catalyzes degradation of cholesterol esters with various chain length acids

Table 3. Means and standard deviations (SD) for lysosomal enzymes activities (nmol/mg of protein/h) in the liver of mice injected over 10 days with morphine hydrochloride

Enzyme	Control groups		Experimental groups					
			20 mg morphine daily per kg body weight			30 mg morphine daily per kg body weight		
	mean	SD	mean	SD	per cent of control	mean	SD	per cent of control
Cath. D and L	7.51	2.00	10.29**	2.10	137	12.62***	1.60	168
AlaAP	8.48	2.49	10.49*	1.67	124	11.56**	0.83	136
LeuAP	8.58	3.17	11.50**	1.74	134	13.52***	4.18	157
LL	7.34	2.18	5.35*	0.50	73	3.01***	0.76	41
β-Glu	1.58	0.66	2.02*	0.38	128	1.92*	0.31	122

*, **, ***Statistically different from control at P≤0.05, P≤0.01 and P≤0.001, respectively.

Table 4. Means and standard deviations (SD) for lysosomal enzymes activities (nmol/mg of protein/h) in the kidneys of mice injected over 10 days with morphine hydrochloride

Enzyme	Control groups		Experimental groups					
			20 mg morphine daily per kg body weight			30 mg morphine daily per kg body weight		
	mean	SD	mean	SD	per cent of control	mean	SD	per cent of control
Cath. D and L	6.53	1.20	8.55**	1.79	131	10.09***	2.27	155
AlaAP	7.70	1.30	9.28*	1.66	121	10.87***	1.83	141
LeuAP	7.22	1.36	9.89**	2.62	137	10.74***	1.37	149
LL	7.17	0.83	5.45*	0.89	76	2.56***	0.41	36
β-Glu	1.56	0.38	1.87*	0.36	120	1.86	0.25	119

*, **, ***Statistically different from control at P≤0.05, P≤0.01 and P≤0.001, respectively.

as well as of other steroids with fatty acids [Du *et al.* 2001]. Our results presented here seem to confirm earlier data of Selevich and Lelevich [1997] suggesting that long-running morphine administration (7 days) in doses increasing from 20 to 40 mg/kg BW may reduce the level of total lipids and cholesterol in cerebral cortex of rats. As LDL-fraction cholesterol is released from lysosomes, where it is hydrolyzed by LL, lysosomal esterases (EL) and phospholipases A_2 [Du *et al*. 2001], we can suppose that morphine used in the present experiment inhibited lipolysis both in liver and in kidneys, what came out with decrease of the examined activity of lysosomal lipase.

Presence of β-Glu has been demonstrated in organs of all species of vertebrates. It catalyzes hydrolysis of terminal β- (1→6) and β- (1→4) glycosidic linkage, containing only carbohydrate chain and participating in transglycosylation [Brazdova *et al*. 2008, Hays *et al*. 1998]. Affected by morphine, the activity of β-Glu increased in all cases studied, but significantly in liver after 30 mg/kg BW dose given over 4 and 10 days

Table 5. Means and standard deviations (SD) for lysosomal enzymes activities (nmol/mg of protein/h) in the liver of mice injected over 14 days with morphine hydrochloride

Enzyme	Control groups		Experimental groups					
			20 mg morphine daily per kg body weight			30 mg morphine daily per kg body weight		
	mean	SD	mean	SD	per cent of control	mean	SD	per cent of control
Cath. D and L	7.45	2.09	9.24*	1.58	124	9.53*	1.46	128
AlaAP	8.19	1.83	9.65	1.40	118	9.27	1.26	113
LeuAP	8.47	1.28	9.82	2.49	116	10.04	1.28	119
LL	7.23	0.67	6.34	1.19	88	7.14	1.16	99
β-Glu	1.63	0.31	2.04*	0.43	125	1.90	0.24	117

*Statistically different from control at P≤0.05.

Table 6. Means and standard deviations (SD) for lysosomal enzymes activities (nmol/mg of protein/h) in the kidneys of mice injected over 14 days with morphine hydrochloride

Enzyme	Control groups		Experimental groups					
			20 mg morphine daily per kg body weight			30 mg morphine daily per kg body weight		
	mean	SD	mean	SD	per cent of control	mean	SD	per cent of control
Cath. D and L	6.86	1.44	8.30*	0.43	121	8.58*	2.19	125
AlaAP	7.55	1.76	9.06*	2.07	120	7.67	0.82	102
LeuAP	7.19	1.91	8.49	0.31	118	8.41	2.16	117
LL	6.97	1.14	5.92	0.59	85	6.76	1.19	97
β-Glu	1.65	0.32	1.97	0.36	119	1.72	0.47	104

*Statistically different from control at P≤0.05.

(Tab. 3 and 4), after 20 mg/kg BW dose given over 10 and 14 days (Tab. 3 and 5), as well as in kidneys after 20 mg morphine/kg BW dose given for 4 and 10 days and after 30 mg/kg BW dose given for 4 days (Tab. 2 and 4). It may be assumed that morphine's metabolites, M3G and M6G are hydrolyzed among other things by β-glucuronidase (β-GlcUr) and by β-Glu [Chen *et al*. 2003]. Thus, the hypothesis, that the growth of activity of the enzyme in question after morphine administration is a definite consequence of its action, seems to be justified.

The results of this study show that morphine used in doses of 20 and 30 mg/kg BW can, apart from the analgesic action, act as a factor mobilizing the rate of metabolism. This may mean that morphine mobilizes also the energy conversion in the organism. Thus, morphine may appear as one of the regulators of metabolism. Change range of lysosomal hydrolases after injections of morphine seems to be interesting, not only from the biochemical, but also from medical point of view. Analogically

to observations of human medicine, the increased time of morphine administration has given the risen resistance to its effects. The obtained data seem to confirm that morphine as specific analgesic agent can increase a rate of metabolic processes in the observed tissues of liver and kidney, affecting the energetic processes in the animal (or human) cells.

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Aktywność enzymów lizosomowych w wątrobie i nerkach myszy poddawanych iniekcjom morfiny

S t r e s z c z e n i e

Badania przeprowadzono na 90 samcach myszy linii Swiss. Zwierzęta podzielono na 9 grup (n=10) – trzy kontrolne (I-III), którym podawano roztwór 0,9% NaCl w okresie 4, 10 i 14 dni oraz sześć grup doświadczalnych (A-F). Myszom grup doświadczalnych A, C i E podawano domięśniowo chlorowodorek morfiny w dawce 20 mg/kg masy ciała przez 4, 10 i 14 dni, a myszom grup B, D i F w dawce 30 mg/kg masy ciała, w analogicznych okresach. Roztwory kontrolne i doświadczalne podawano w iniekcji domięśniowej, raz dziennie między godziną 9:00 a 10:00. W lizosomowych supernatantach wątroby i nerek oznaczano aktywność kateptyczną (katepsyna D i L), aminopeptydazy alaninowej, aminopeptydazy leucynowej, lipazy lizosomowej i β-glukozydazy. Iniekcje chlorowodorku morfiny spowodowały wzrost aktywności badanych enzymów w wątrobie i w nerkach, z wyjątkiem lipazy lizosomowej, której aktywność uległa obniżeniu.