

Dexamethasone antagonizes morphine effects on GSSG levels

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Abstract

Dexamethasone (DEX) is a highly potent logacting glucocorticoid with negligible sodium retaining properties. Recent evidence has been obtained for an interference caused by DEX on morphine induced analgesia, hypermotility, constipation and contraction of guinea pig isolated ileum. Studies in our laboratory have recently focused on the relationship between morphine and glutathione (GSH) levels.

The present study was carried out to determine whether DEX may affect the morphine induced GSH variation in the brain. Male albino Wistar rats (180-200 g) received i.p. morphine (100 mg/kg), DEX (10 mg/kg) and were sacrificed 3 hours after the administration, in the brain GSH and glutathione disulfide (GSSG) were determined by an automatic procedure developed in our laboratory. The results show that DEX is not able to inhibit the GSH levels induced by morphine but completely counteracts the effect on GSSG levels (nmoles/g tissue). In fact, the GSSG for the control group was found to be 2.8 ± 0.56 for the morphine treated group was 5.7 ± 0.46 for the DEX treated group was 3.0 ± 0.49 and for the DEX+Morphine treated group was 3.0 ± 0.48 .

The present study shows that DEX is also able to interfere with morphine induced GSH variation.

Introduction

It is widely reported that hepatic toxicity induced by morphine in rat is related to its metabolites rather than morphine alone [1-7]. These metabolic products are able to induce free radicals and/or binding with glutathione (GSH), the natural scavenger of superoxide radicals.

GSH is one of the most important intracellular thiolic compounds which is involved in several and fundamental cellular functions.

Both GSH conjugation and its subsequent depletion cause a free radicals accumulation as well as morphine metabolites induce directly and indirectly cellular toxicity [8] with enzymatic inactivation, DNA damage and/or lipidic peroxidation (Fig. 1).

In order to verify the presence of such phenomena in brain, we have measured in the rat reduced and oxidized glutathione levels (GSSG) after morphine administration.

Moreover, since it was reported that DEX influences significantly morphine effects [9-13], we have also verified if this glucocorticoid is able to act on morphine-GSH model.

Lipid detoxification
GSH oxidation and depletion
Protein thiolic compound oxidation
Homeostasis ionic alteration
Harm to DNA
Cytoskeletal alterations
Mitochondrial alterations
ATP depletion
Enhancing of permeability of plasmatic cell membrane



cellular death

Fig. 1: Multifactor of cellular harm by oxidative stress

Materials and Methods

For our experiments, we used albino Wistar male rats (average weight 200g) kept in standard conditions of temperature (21°C) and diet (standard diet: MIL, Morini, S.Polo d'Enza, Italy). The animals had free access to water

The animals were divided in 3 groups which received through i.p.:

- a solution of morphine 15,20, 50 and 100 mg/Kg;
- a solution of DEX 10 mg/Kg;
- concurrent administration of morphine 100 mg/Kg and DEX 10 mg/Kg.

The control group which received through i.p. administration only saline solution in the same quantity of the corresponding group of rats which didn't have any treatment were considered "white".

Animals were sacrificed by decapitation always at the same hour of the day (4 P.M) to avoid the influence of circadian rhythm for GSH determination. After sacrifice, the brain was quickly removed and immediately dissected on a cold surface (0°C) and homogenized at pH=4 phosphate buffer (1:3 w/v).

Homogenates were used to determine:

- GSH by the Tietze's method, by a procedure automated in our laboratory [15];
- GSSG with Griffith method [16];
- Proteins in accordance with Lowry method [17]

No statistical differences were observed among the control groups and therefore they were collected in a single pool.

Results and Discussion

The results are reported in Tables 1-4. Morphine at the doses used (15, 20, 50 and 100 mg/Kg/ip) did not induce per se a significative variation of total GSH in the rat brain (Table 1).

This observation may appear in contrast with previous data obtained with hepatic GSH [18] where total GSH depletion is significant and dose dependent. This result may be explained considering that the reactive metabolites do occur only in the liver for oxidation by cytochrome P450 and that they are not transported in the brain (or only partially). Therefore, the content of total GSH could not receive a diminution proportional to the dose of morphine administered, considering also the variability of its metabolism [19].

By contrast, it was observed that morphine increased significantly and dose-dependently the GSSG levels (Table 2).

DEX alone did not induce any significant alterations both in GSH and GSSG levels (Tables 3 and 4) whereas it was able to reduce the morphine induced increase in GSSG levels (Table 4).

The results of the present study show a further interaction between DEX and morphine thus confirming our previous studies [8-12]. Although, the mechanism underlying this interaction is still unclear, we may suggest a hypothesis. One possibility may be due to a mechanism linked to Ca²⁺ homeostasis. It is known that glucocorticoids increase the uptake of Ca²⁺, establishing the altered balance caused by the increase of intracellular Ca²⁺ after morphine administration [21].

The return in Ca²⁺ balanced conditions and in the consequent regular membrane permeability may have a key role in the modulation of "oxidative stress" shown by the abnormal enhance of brain GSSG levels found after morphine administration (100 mg/kg i.p.).

These results may also explain intracellular Ca²⁺ increase, with immediately hyperpolarization of cellular membrane, showed after morphine administration [21].

Also, we observed a significant increased of GSSG/GSH ratio with a corresponding reduction of NADPH levels and a modification of Ca²⁺ compartmentalization (Figure 2) [20-22].

Table 1: Effect of morphine on total glutathione levels (GSH)

Group	Dose mg/Kg	n	GSH m	S.D.
control	-	16	1.8	0.27
morphine	15	12	1.5	0.19
	25	11	1.5	0.34
	50	7	1.4	0.26
	100	7	1.3	0.26

Results are expressed as nmol/g of tissue in rat brain.
n: animals number; m: media; s.d.: standard deviation

Table 2: Effect of morphine on glutathione oxidized levels (GSSG)

Group	Dose mg/Kg	n	GSSG m	S.D.
control	-	14	2.7	0.62
morphine	15	12	4.0	0.67
	25	11	4.4	0.66
	50	7	4.9	0.64
	100	7	5.6	0.47

Results are expressed as μmol/g of tissue in rat brain. n: animals number; m: media; s.d.: standard deviation.

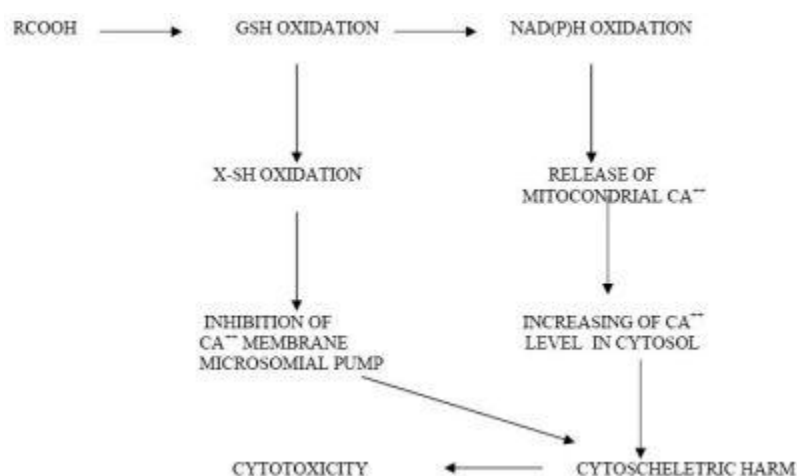
Table 3: Effect of morphine (M; 100 mg/Kg), dexamethasone (DEX; 10 mg/Kg) on total glutathione levels (GSH).

Group	Dose mg/Kg	n	GSH m	S.D.
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control	-	16	1.8	0.27
M	100	7	1.3	0.26
DEX	10	10	1.8	0.31
DEX+M	10+100	10	1.2	0.42

Results expressed as nmol/g of tissue in rat brain. n: animals number; m: media; s.d.: standard deviation.

Fig. 2: Alteration of calcic homeostasis during hyperoxides methabolism. Possible sequences of events.



(For larger image, click [here](#))

Table 4: Effect of morphine (M; 100 mg/Kg), dexamethasone (DEX; 10 mg/Kg) on glutathione oxidized levels (GSSG)

Group	Dose mg/Kg	n	GSSG m	S.D.
control	-	14	2.7	0.62
M	100	7	5.6	0.47
DEX	10	9	3.0	0.49
DEX+M	10+100	9	3.0	0.48

Results are expressed as nmol/g of tissue in rat brain. n: animals number; m: media; s.d.: standard deviation.

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