Stress by restraining potentiates morphine catalepsy in rats

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Summary. Restraint-induced stress potentiated morphine catalepsy in rats. This potentiation was partially antagonized by pharmacologic treatments decreasing central serotonin, acetylcholine, prostaglandins and by naloxone. Selective increase in central dopamine also inhibited the potentiation.
Key words. Immobilization-induced stress; morphine catalepsy; rat brain; serotonin; acetylcholine; prostaglandins; dopamine; naloxone.

Catalepsy in laboratory animals, is defined as the inability to correct externally imposed postures and is known to be induced by neuroleptic and a large number of non-neuroleptic agents, including morphine[2]. Central neurotransmitter mediation of morphine catalepsy has been extensively studied[3]. Restraint-induced stress was shown to alter the thermic response of morphine in rats[4]. Some experimental influences, including handling, have been reported to affect morphine catalepsy[5]. Recent studies indicate that restraint-induced stress potentiates haloperidol catalepsy in rats[6]. In the present communication we report the effect of restraint stress on morphine catalepsy and the possible mechanism of the stress-morphine interaction.

Materials and methods. Male Wistar strain albino rats (120–180 g) were used. The rats were housed in colony cages at an ambient temperature of 25 °C, with a 12 h light, 12 h dark cycle. Experiments were conducted at this temperature between 09.00 and 14.00 h. Food was withdrawn 18 h prior to and water before the experimentation. Stress was induced by immobilization in adjustable metal restraint chambers[7] for 1, 2 or 4 h. Catalepsy was quantiated by the 'ring test'[8], modified for use in rats[3]. After termination of the specified period of restraint, a predetermined subcataleptic dose (1 mg/kg, i.p.) of morphine sulphate was administered and the depth of catalepsy was determined after 15 min when the peak cataleptic effect of morphine is usually achieved[3]. The rat was placed on a steel ring (diameter 12 cm) fixed to a steel stand at a height of 40 cm. The time during which the rat remained completely immobile, with total cessation of snout and whisker movements, during an observation period of 5 min, was converted into 'percent immobility[8]. Since repeated testing and handling of the experimental animals are known to progressively increase cataleptic scores[5], naive rats were used each time and the control animals were handled is a similar manner to the rats in the experimental groups.

The following drugs, with abbreviations, doses and pretreatment times given in parenthesis were used: 5,6-dihydroxytryptamine (DHT, 75 µg/rat, 48 h) p-chlorophenylalanine (PCPA, 100 mg/kg, once daily for 3 days), hemicholinium-3 (HC, 20 µg/rat, 45 min), diclofenac sodium (20 mg/kg, 2 h), mefenamic acid (25 mg/kg, 1 h), naloxone (1 mg/kg, 1 h), L-dopa (100 mg/kg, 30 min) with benserazide (50 mg/kg, 30 min) in diethyldithiocarbamate sodium (DDC, 250 mg/kg, 3 h) treated rats. All the drugs were administered i.p., dissolved or suspended in 0.5 ml of normal saline with the exception of DHT and HC, which were administered intracerebroventricularly (i.c.v.), dissolved in 10 µl of artificial cerebrospinal fluid (CSF), through indwelling cannulae inserted stereotaxically into the right lateral ventricle. Control rats received equivalent volumes of normal saline or artificial CSF as per the experimental protocol. The doses and pretreatment times of the drugs used are based on earlier studies from this laboratory[3,6]. None of the pharmacologic agents used to determine the mechanism of stress-morphine interaction had any per se cataleptic effect in the doses used, as indicated by earlier studies[3,6]. Statistical evaluation was done by the Student's t-test.

Results and discussion. The results are summarized in the table. Since the two vehicles used, artificial CSF (i.c.v.) and normal saline (i.p.), produced minimal cataleptic effects not significantly different from each other, their values have been pooled together. As is evident from the data, morphine produced a dose-related cataleptic response with minimal catalepsy being induced by the dose of 1 mg/kg. Hence this dose was used in the present study as the subcataleptic dose of morphine. Restraint stress (1, 2 and 4 h) did not induce per se catalepsy, as has been reported earlier[6], but significantly potentiated the catalepsy induced by morphine. Maximal potentiation was afforded by 1 h restraint, whereas immobilization for 2 and 4 h was found to induce progressively lesser degrees of potentiation.

Restraint (1 h)-induced potentiation of morphine catalepsy was significantly attenuated after pretreatment with pharmacologic agents known to decrease central serotonin, namely, DHT, which induces selective degeneration of serotonergic neurones, and PCPA, a specific inhibitor of tryptophan hydroxylase. Similarly, HC, which decreases central acetylcholine (Ach) by inhibiting the uptake of choline into the cholinergic neurones, also inhibited the potentiation. Likewise, the prostaglandin (PG) synthesis inhibitors, diclofenac and mefenamic acid, and naloxone, an opioid receptor antagonist, significantly antagonized restraint stress-morphine interaction. Selective increase in central dopamine (DA), induced by giving L-dopa with a peripheral decarboxylase inhibitor, benserazide, in rats primed with DDC, a DA-β-hydroxylase inhibitor, also attenuated this potentiation. The 'ring test' was used to quantitate catalepsy because it has been shown to be sensitive enough for the bioassay of cannabinoids[8] and has been extensively used in this laboratory[3,6]. The results indicate that reduction in central serotonin, Ach, PG and

Effect of restraint-induced stress on morphine catalepsy and the effects of some drugs on stress-morphine interaction in rats

Groups	Percent immobility		
	n	Mean ± SEM	p
Control (vehicle)	26	8.6 ± 3.8	—
Morphine – 1 mg/kg, i.p. (M)	10	15.3 ± 2.8	$> 0.05^a$
Morphine – 2 mg/kg, i.p.	10	32.3 ± 3.6	$< 0.001^a$
Morphine – 5 mg/kg, i.p.	10	56.5 ± 3.9	$< 0.001^a$
Morphine – 10 mg/kg, i.p.	10	77.3 ± 2.4	$< 0.001^a$
Restraint – 1 h + Saline	6	10.2 ± 4.2	$> 0.05^a$
Restraint – 2 h + Saline	6	12.6 ± 3.9	$> 0.05^a$
Restraint – 4 h + Saline	6	12.9 ± 3.2	$> 0.05^a$
Restraint – 1 h + M (RM)	10	66.4 ± 5.2	$< 0.001^b$
Restraint – 2 h + M	10	52.6 ± 3.9	$< 0.001^b$
Restraint – 4 h + M	10	34.1 ± 4.9	$< 0.01^b$
DHT + RM	6	30.9 ± 4.0	$< 0.001^c$
PCPA + RM	6	48.2 ± 2.1	$< 0.01^c$
HC + RM	6	32.5 ± 4.6	$< 0.001^c$
Diclofenac + RM	6	45.0 ± 4.2	$< 0.01^c$
Mefenamic acid + RM	6	42.3 ± 3.9	$< 0.01^c$
Naloxone + RM	8	31.9 ± 4.2	$< 0.001^c$
L-Dopa (with DDC and benserazide) + RM	8	36.3 ± 5.2	$< 0.01^c$

[a–c] indicate statistical significance in comparison to Control, Morphine (1 mg/kg) and the respective saline-treated restraint group, and Restraint (1 h) stress + Morphine (1 mg/kg) groups, respectively.
The pharmacologic agents used to investigate stress-morphine interaction had no significant effect on the experimental parameter[3,6].

endogenous opioid activity attenuates restraint stress-induced potentiation of morphine catalepsy, as also does an increase in central DA activity. Since restraint stress-morphine interaction appears to involve several neurotransmitter systems it is cogent to explain it in terms of a central neuromodulator system which could induce the observed changes. PGs have been postulated to function as modulators of central synaptic transmission, and have been proposed as the first mediator of stress[9]. Restraint stress has been reported to enhance rat brain PG levels[10]. PGs of the E series have been shown to enhance rat brain serotonin[11] and cholinergic[12] activity, and to inhibit the release of DA from the rat striatum[13]. Recent studies indicate that PGD_2, the major rat brain PG, also enhances central serotonergic activity in this species[14]. Restraint stress-induced increase in rat brain serotonin has been shown to be antagonized following pretreatment with PG synthesis inhibitors[15]. While literature on the inter-relationship between central opioid and PG systems is sparse, it is known that naloxone inhibits the increase in rat brain PGs induced by met-enkephalin[16]. In a recent study[17], PGE_1-induced catalepsy in rats was shown to be attenuated following treatments disigned to reduce central serotonin and Ach, and to specifically enhance DA levels. Naloxone also antagonized PGE_1 catalepsy.

Experimental catalepsy is known to result from an interplay between central neurotransmitter systems. Thus, a decrease in central DA activity and an increase in brain serotonergic and cholinergic activities has been envisaged as the primary mechanism of experimental catalepsy[2,18]. It appears likely that PGs, released during restraint stress, potentiate morphine catalepsy by enhancing rat brain serotonin[11,14] and Ach[12], and reducing DA[13] activity. This is indicated by the attenuating effect of the PG synthesis inhibitors on stress-morphine interaction. Treatments which reduce central serotonin or Ach and enhance DA levels would then be expected to partially antagonize restraint stress-induced potentiation. Experimental stress is known to enhance rat brain endorphin level[19]and the inhibition induced by naloxone on stress-morphine interaction is in keeping with this observation.

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Ethanol metabolizing system in *Drosophila melanogaster:* subcellular distribution of some main enzymes

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Summary. The subcellular distribution of some enzymes which play a part in ethanol metabolism have been determined by differential centrifugation of homogenates of adult *D. melanogaster* flies of various genotypes. Aldehyde dehydrogenase, recently discovered in *D. melanogaster*, is present in the five genotypes studied. It has been found however to be, in vitro at least, most active in a strain lacking both alcohol dehydrogenase and aldehyde oxidase.
Key words. D. melanogaster; aldehyde dehydrogenase; catalase; alcohol dehydrogenase; aldehyde oxidase; ethanol metabolism.

Flies of five *D. melanogaster* genotypes were submitted by us to homogenization and subcellular fractionation according to a method originally described for rat liver[3], and slightly modified[4]. Five fractions were isolated. A nuclear fraction (N) was first separated from a total cytoplasmic extract (E). Then from the cytoplamic extract, four fractions were isolated: a heavy mitochondrial fraction (M), a light mitochondrial fraction (L), a microsomal fraction (P), and a final supernatant (S). Our main interest was in alcohol dehydrogenase (ADH), catalase, aldehyde oxidase (AO), and aldehyde dehydrogenase (ALDH). The enzymatic activities were determined according to Sofer and Ursprung for ADH[5], to Baudhuin et al. for Catalase[6], to Courtright for AO[7], and to Crow et al.[8] and Eckfeldt et al.[9] for ALDH. We used cytochrome *c* oxidase[10] and malate dehydrogenase[11] as marker enzymes for the mitochondria, while acid phosphatase[3] and beta-galactosidase[12] were used for lysosomes, and NADPH cytochrome *c* reductase[13] was the marker enzyme for endoplasmic reticulum. In the case of peroxisomes, catalase was the reference enzyme[5].

Besides the *bAdh*^{n4}, used in our previously described experiments[4], which lacks both ADH and AO, four other *D. melanogaster* genotypes were examined. The strain *y v f mal*^{bz} (Johns Hopkins University, Dept of Biology), lacks AO but has a normal ADH activity. Both HA and LA lines are the result of a long term selection for the 'male sexual activity', combined with brother-sister mating, which has given, after 330 generations, a 'highly active' line HA and a 'lowly active' line LA[14]. As in our previous experiments[15-17], our laboratory *wild* $e^{\pm}$ strain was used