

SHORT COMMUNICATION

EFFECT OF RESTRAINT STRESS ON CANNABIS-INDUCED CATALEPSY  
IN RATS

S. K. BHATTACHARYA AND P. GHOSH

*Department of Pharmacology,  
Institute of Medical Sciences, Banaras Hindu University, Varanasi - 221 005*

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**Summary :** The effect of restraint stress (1, 2 and 4 hr) on cannabis-induced catalepsy, was investigated in rats. Restraint stress produced a time-related potentiation of the cataleptic effect of a sub-cataleptic dose of cannabis. Stress (4 hr)-induced potentiation of cannabis catalepsy was attenuated after pretreatment of the animals with drugs known to decrease central 5-HT and prostaglandin activity, but was unaffected by metyrapone, an inhibitor of endogenous corticoid synthesis. The results suggest the involvement of 5-HT and prostaglandins in restraint stress-cannabis interaction. The results have been discussed in the light of earlier investigations, from this laboratory, indicating increased rat brain 5-HT and prostaglandin activity, following restraint stress, and possible 5-HT mediation in central effects of prostaglandins. It is suggested that restraint stress first enhances rat brain prostaglandins, which in its proposed role as the 'first mediator' of stress, activates the serotonergic system in this species. This prostaglandin 5-HT link, thus, mediates the observed potentiating effect of restraint stress on cannabis catalepsy.

**Key words :** restraint stress                      cannabis catalepsy                      prostaglandins  
5-HT    corticoids

INTRODUCTION

The behavioural effects of cannabis in rats are known to be dependent on the physiological state of the animal. Thus, contrary to its usual depressant effect in normal rats, cannabis induces a state of irritability and aggressiveness in animals previously stressed by chronic starvation, cold or deprivation of sleep (1, 11). In sleep-deprived rats, cannabis induces long aggressive episodes intercalated by periods of a catatonia-like state (11). Cannabis and its surrogates are known to induce catalepsy in experimental animals, including rats and mice (13, 16), and in man (17).

A variety of experimental stressors are known to affect central neurotransmitters (21). However, it is not clear as to which of these putative neurotransmitters function as the 'first mediator' of stress, responsible for alerting the animal to the existence of increased demand upon its physiological system, induced by the stressor (21). Despite the well accepted effects of experimental stress on central neurotransmitters, little attention has been paid to the effect of stress on the pharmacological actions of centrally acting drugs, most of them being known to be mediated through one or more of these transmitters. Recent reports from this laboratory have shown that restraint stress induces auto-analgesia and potentiates hexobarbitone hypnosis in rats (5, 7). Both these stress effects were shown to be prostaglandin (PG) and 5-hydroxytryptamine (5-HT) mediated responses.

### MATERIALS AND METHODS

The studies were conducted on male Wistar strain rats (100-150 g), maintained on standard Hind Lever diet and housed in colony cages in an air cooled ( $25 \pm 2^\circ\text{C}$ ) room. Experiments were conducted at this ambient temperature between 9 a.m. and 2 p.m. Food was withdrawn 18 hr prior to and water just before experimentation. Restraint stress was induced by tying fore and hind limbs separately and then together, and finally immobilising the rats in adjustable metallic restraint chambers (21).

Catalepsy was quantitated by the ring test (18), modified for use in rats (9). After the termination of the period of restraint (1, 2 or 4 hr), a previously determined sub-cataleptic dose (50 mg/kg, ip) of cannabis resin was administered and the depth of catalepsy was determined 1 hr later. 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, out of a total observation period of 5 min, was converted into percent immobility. Sometime the rat jumped off or fell from the ring before 5 min. If it had remained on the ring for more than 2.5 min, the percent immobility was calculated for this reduced time period. However, if the rat jumped off the ring successively for five times before 2.5 min, the animal was discarded (18).

Cannabis resin (CI) was extracted from the flowering tops of *Cannabis indica* with petroleum ether (60-80°C) and was suspended in 1% Tween-80 for experimentation. Delta-9-tetrahydrocannabinol (THC) content of the resin was biologically assayed

by the 4-point assay technique, using pure THC as standard and taking hypothermic activity in rats as the assay parameter. The THC content of the resin was 15%.

The following drugs, with dose and pretreatment time given in parentheses, were used to investigate restraint stress effects on CI catalepsy: 5,6-dihydroxytryptamine (DHT, 75  $\mu$ g/rat, 48 hr), p-chlorophenylalanine (PCPA, 100 mg/kg, once daily for 3 days), methysergide (5 mg/kg, 1 hr), cyproheptadine (5 mg/kg, 30 min), diclofenac (15 mg/kg, 6 hr), mefenamic acid (50 mg/kg, 30 min), paracetamol (100 mg/kg, 30 min) and metyrapone (20 mg/kg, two injections at 8 and 4 hr). The doses refer to the respective salts and the pretreatment time indicates the time interval between drug administration and induction of restraint. All the drugs were given ip, except DHT, which was administered intra-cerebroventricularly, dissolved in artificial cerebrospinal fluid. Control rats received equivalent volumes of 1% Tween-80, the vehicle for CI.

Statistical evaluation was done by the Student's *t* test.

## RESULTS

The results are summarized in Table I. CI (50,100 and 200 mg/kg, ip) produced a dose-related cataleptic response. Since CI (50 mg/kg) produced minimal catalepsy, *per se*, it was used as the sub-cataleptic dose of CI for further experimentation. Restraint stress (1, 2 and 4 hr) did not show any cataleptic effect, whatsoever, but produced a time-related potentiation of CI catalepsy, the effect being significant at 2 and 4 hr. Restraint stress (4 hr)-induced potentiation of CI catalepsy was significantly attenuated after pretreatment with DHT, PCPA, methysergide, cyproheptadine, diclofenac, mefenamic acid and paracetamol, to the extent of 61.4%, 55.4%, 33.2%, 41.7%, 43.3%, 35.7% and 51.5%, respectively. Metyrapone produced insignificant inhibition (5.3%).

## DISCUSSION

Among the many methods for assessing cataleptic effect of drugs, the ring test was chosen because it has been shown to be sensitive enough for bioassay of cannabinoids (18). Restraint stress produced a time-related potentiation of the cataleptic effect of a sub-cataleptic dose of CI, though it was bereft of any cataleptic effect *per se*. Stress-induced (4 hr) potentiation of CI catalepsy was selected for detailed studies. Pretreatment with DHT, a selective central 5-HT neuronolytic agent (2), PCPA, a specific 5-HT synthesis inhibitor (15), and the 5-HT receptor antagonists, methysergide and cyprohep-

tadine, markedly attenuated restraint stress (4 hr)-induced potentiation of CI catalepsy. The prostaglandin (PG) synthesis inhibitors (12), diclofenac, mefenamic acid and paracetamol, also inhibited the potentiation. Paracetamol is known to be a selective inhibitor of brain PG synthesis (12). On the contrary, metyrapone, which inhibits endogenous corticoid synthesis (22) failed to affect stress-induced potentiation of CI catalepsy. The results, thus, indicate that pharmacological treatments which reduced brain PG and 5-HT activity interfere with CI potentiation by restraint stress. Stress-induced activation of endogenous corticoid activity does not appear to be involved.

TABLE I : Effect of restraint stress (1, 2 and 4 hr) on the cataleptic effect of a sub-cataleptic dose of cannabis resin, and the effects of the experimental drugs on restraint (4 hr)-induced potentiation of cannabis catalepsy in rats.

Groups	n	Percent immobility Mean $\pm$ S.E.	P
Control (Tween-80, 1%)	10	9.5 $\pm$ 3.2	—
Cannabis (50 mg/kg)	10	16.2 $\pm$ 2.9	>0.05*
Cannabis (100 mg/kg)	10	47.6 $\pm$ 3.2	<0.01*
Cannabis (200 mg/kg)	10	82.8 $\pm$ 1.6	<0.001*
Restraint (1 hr) +Cannabis (50 mg/kg)	10	32.0 $\pm$ 3.7	>0.05**
Restraint (2 hr) +Cannabis (50 mg/kg)	10	59.2 $\pm$ 3.4	<0.01**
Restraint (4 hr) +Cannabis (50 mg/kg) (R-CI)	10	81.6 $\pm$ 2.2	<0.001**
DHT+R-CI	6	31.5 $\pm$ 2.7	<0.001*
PCPA+R-CI	10	39.4 $\pm$ 3.3	<0.001*
Methysergide+R-CI	10	54.5 $\pm$ 2.9	<0.001*
Cyproheptadine+R-CI	10	48.4 $\pm$ 3.7	<0.001*
Diclofenac+R-CI	10	46.3 $\pm$ 4.2	<0.001*
Mefenamic acid+R-CI	10	52.5 $\pm$ 3.9	<0.001*
Paracetamol+R-CI	10	39.6 $\pm$ 4.0	<0.001*
Metyrapone+R-CI	6	77.3 $\pm$ 2.9	>0.05*

\*, \*\* and \* indicate statistical significance in comparison to control (Tween-80), cannabis (50 mg/kg) and restraint (4 hr) potentiated cannabis (50 mg/kg) group (R-CI), respectively (t-test).

Cannabis-induced catalepsy in mice has recently been reported to be a 5-HT mediated response (13). There are no reports on the possible effect of PGs on drug-induced catalepsy, apart from a solitary communication which indicates that cannabis-induced catalepsy, in mice, is potentiated by  $\text{PGE}_1$  and is inhibited by  $\text{PGF}_2\alpha$  and PG synthesis inhibitors (6). PGs have been known to induce catalepsy in rats, in high doses (20). Restraint stress has been shown to enhance rat brain 5-HT activity (4) and enhance PG levels in this species (3).

PGs have been suggested to be the 'first mediator' of stress, since they are released in response to noxious stimuli and can activate the hypothalamo-hypophyseal-adrenal axis, directly or indirectly (14). 5-HT is regarded as essential for maintaining physiological and psychic homeostasis during stress (19). Recent studies from this laboratory suggest that PGs modulate central 5-HT activity in rats (8). Keeping the earlier mentioned (3,4) biochemical effects of restraint stress in view, it is conceivable that the observed potentiation of CI catalepsy in restrained rats, is a consequence of this PG modulated enhanced 5-HT activity, activated by stress. 5-HT mediation of cannabis-induced catalepsy in mice and the enhancement of rat brain 5-HT activity by cannabis (9), together with the afore-mentioned PG effects on cannabis-induced catalepsy (6), support such a contention.

The ineffectiveness of metyrapone, an inhibitor of endogenous corticoid synthesis (22), in restraint stress-CI interaction, not only rules out a role for peripheral corticoids, but also supports the above mentioned PG-5-HT link in the potentiating effect of restraint stress, albeit indirectly. Bilateral adrenalectomy and metyrapone have been shown to have insignificant effects on restraint stress induced increase in rat brain 5-HT (4) and PG (3) activity.

Restraint stress has been shown to enhance brain penetration of barbiturates in rats (10). It is not known whether a similar facilitation of the passage of CI across the blood-brain barrier, occurs after restraint stress. The present study does not negate this possibility. However, the findings are more consistent with a PG-5-HT mediated response in the rat brain.

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