Animals

Albino mice (Laka strain) or rats (Porton strain), bred in Central Animal House facility of Panjab University and weighing 20-25 g or 200-250 g, respectively were used. The animals were selected at random, irrespective of sex except in studies (vi and vii) involving measurement of nociceptive sensitivities where only male mice were used. The animals were housed under standard 12 hr light/12 hr dark cycle with food (Hindustan Lever Products, Calcutta, India) and water provided ad libitum, both being withdrawn 12 hr prior to experimentation. The experiments were performed between 9.00 and 17.00 hrs.

Drugs

Apomorphine (Sigma, St.-Louis, MO, USA), B-HT 920 [2-amino-6-allyl-5, 6, 7, 8-tetrahydro-4H-thiazolo-(4, 5-d)-azepine hydrochloride] (Talipexol; Boehringer-Ingelheim, Ingelheim am Rhein, Germany), clozapine (Sandoz, Switzerland), clonidine hydrochloride (Boehringer-Ingelheim, Ingelheim am Rhein, Germany), cyproheptadine (Merck, Sharp and Dohme, Rahway, NJ, USA), dopamine hydrochloride (TTK Pharmaceuticals, Madras, India), idazoxan (Reckitt & Colman, Hull, England), ketamine (Themis Chemicals Ltd., Bombay, India), molindone (Endo Laboratories, NY, USA), morphine (Government Analytical Laboratory, Chandigarh, India), MK 801 [5-methyl -10, 11-dihydro-5H-dibenzo (a,d) cyclohepten-5, 10-imine] (dizocipline; Merck, Sharp and Dohme,
Rahway, NJ, USA), naloxone (Endo Laboratories, NY, USA), pentobarbitone sodium (John Bake Inc., USA) perphenazine hydrochloride (Schering Co., Kenilworth, NJ, USA), rimcazole (Wellcome Research Laboratories, NC, USA), SKF 38393 [1-phenyl-2, 3, 4, 5-tetrahydro-(1H)-3-benzazepine-7, 8-diol hydrochloride] (Research Biochemicals Inc., Natick, MA, USA), SCH 23390 [R(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol hydrochloride] (Schering Plough Co., Bloomfield, NJ, USA), (-)-sulpiride (Delagrange, Paris, France) and scopolamine hydrobromide (Merck & CO., Inc., NJ, USA) were dissolved in deionized water. Reserpine (Loba Chemicals, Bombay, India) was dissolved in a few drops of glacial acetic acid and diluted with deionized water. Haloperidol (Searle, Skokie, IL, USA) and bromocriptine (Sandoz, Switzerland) were dissolved in a few drops of lactic acid, volume made up with deionized water and pH adequately adjusted. Alpha-methyl-p-tyrosine (AMPT; Sigma, St.-Louis, MO, USA) was dispersed in a deionized water/Tween 80 mixture (2 drops/10 ml).

**Statistical analysis**

The data expressed as mean ± SEM was analysed by one-way analysis of variance (ANOVA) followed by Student’s t-test or Duncan’s multiple range test. p<0.05 was considered statistically significant.
DIFFERENTIAL ROLE OF DOPAMINE RECEPTOR SUBTYPES IN THERMOREGULATION AND STEREOTYPIC BEHAVIOUR IN NAIVE AND RESERPINIZED RATS

Recording of rectal temperature

The rats were restrained in a rat restrainer and the variation in rectal temperature was recorded using a telethermometer (Yellow Springs Instrument Co. Inc., Yellow Springs, OH, USA) by inserting the thermistor probe to a depth of 2-3 cm into the rectum. Rectal temperature of each rat was recorded at 0, 15, 30, 45, 60, 90 and 120 min after drug administration. The temperature of each animal was recorded for 1 min. The ambient temperature was 25 ± 0.5°C and the rectal temperature recorded at zero time, served as control for each animal, in addition to a separate corresponding vehicle-treated control group being included in the study.

Measurement of stereotypy

Rats were placed individually in glass containers. Sniffing, rearing, licking, biting, gnawing and grooming were observed as stereotypic behaviours at 0, 5, 10, 15, 30, 45, 60, 90 and 120 min, respectively after drug administration. The intensity of stereotypy was recorded as described by Costall and Naylor (1973). The cumulative stereotypy score was calculated by adding all the scores for the purpose of comparison.

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Drug administration

Various doses of B-HT 920 (0.25 - 1 mg/kg) and apomorphine (0.25-2 mg/kg) were administered. In combination studies, B-HT 920 and SKF 38393 were administered simultaneously. Apomorphine was given 15 min after the administration of B-HT 920 or SKF 38393. The antagonists, namely haloperidol, sulpiride, idazoxan and cyproheptadine, respectively were administered 30 min prior to agonists except SCH 23390, which was given 15 min before the agonists. The selection of doses was based on previous reports from our laboratory. The rats received either a single injection of reserpine (5 mg/kg), 24 hr prior to the experiment (acute reserpinization) or reserpine (1 mg/kg) was administered once daily for the first 4 days, and then once every other day for a total of 15 days (chronic reserpinization). The animals did not receive reserpine treatment on the last day and at least 24 hr elapsed between the last reserpine injection and the equipment. Corresponding vehicles were used for all control injections. All the drug solutions were freshly prepared prior to experimental sessions and administered intraperitoneally (i.p.) in a constant volume of 0.5 ml/100 g body weight. Each group comprised 5-10 rats.
ON THE $D_1$ AND $D_2$ DOPAMINE RECEPTOR PARTICIPATION IN LEARNING AND MEMORY IN MICE

Apparatus

Passive avoidance paradigm

The apparatus consisted of an electric grid (24x30 cm) with a centrally located shock-free zone (SFZ: 2x3x1 cm) and the entire grid having a perflex enclosure (Sharma and Kulkarni, 1990).

Elevated plus-maze

An elevated plus-maze consisting of two open arms (16x5 cm) and two enclosed arms (16x5x12 cm) was used. The maze was elevated to a height of 25 cm (Kulkarni et al., 1991).

Experimental procedures

Passive avoidance learning

The mice were put individually on the electric grid and allowed to explore for 1 min. The stimulus (20 V) was then applied and the latency to reach SFZ was recorded three consecutive times as basal readings. Animals that reached SFZ in 2 min in the first trial were selected for the study.

After 1 hr of training, each animal was put on the grid and the latency to reach SFZ was recorded as a parameter for acquisition. Retention latency was similarly recorded 24 hr after training.
Elevated plus-maze: Measurement of transfer latency (TL)

The method employed to measure TL was essentially the same as described by Itoh et al. (1990). The mice were put individually at the end of one open arm facing away from the centre of the maze and the time the mouse took to move from the arm to either of the enclosed arms was recorded as TL. TL was the time elapsed between the time the animal was placed in the open arm and the time when it fully entered (all the four paws in) the enclosed arm. On the 1st day the mouse was allowed to explore the plus maze for 20 sec after the measurement of TL. The mice were returned to their home cages after the first trial. Twenty four hours later, the mice were placed on the elevated plus-maze individually as before and TL was recorded again. TL measured on 1st and 2nd day served as parameters for acquisition and retrieval, respectively. If the mouse did not enter the enclosed arm within 90 sec on the second trial, TL was assigned to 90 sec.

Drug administration

The drugs were administered, either alone or in combination, after passive avoidance training on the 1st day or prior to the first trial on elevated plus-maze. The antagonists namely haloperidol (0.05 and 0.1 mg/kg), molindone (2.5 mg/kg), clozapine (10 mg/kg) and yohimbine (1 mg/kg) were administered immediately after passive avoidance training or 1 hr prior to recording TL on the 1st
day. SCH 23390 (0.1 mg/kg) was, however, administered 30 min after passive avoidance training or prior to recording TL on the 1st day. B-HT 920 or SKF 38393 was administered, either alone or in combination, 30 min after passive avoidance training or recording of TL on 1st day. Bromocriptine was given 2 hr prior to acquisition test on passive avoidance paradigm as well as elevated plus-maze. Scopolamine was administered 30 min prior to acquisition test, either alone or in combination with B-HT 920 and SKF 38393, respectively. Corresponding vehicles were used for all the control injections. All the drug solutions were freshly prepared prior to experimental sessions and administered i.p. in a constant volume of 1 ml/100 g of body weight. The selection of doses was based on the earlier reports from our laboratory. Each group comprised 5-26 mice.

EFFECT OF D₁ AND D₂ DOPAMINE AGONISTS ON NEOCORTICAL AND HIPPOCAMPAL EEG ACTIVITY OF RAT BRAIN

Electrode implantation and EEG recording

Under pentobarbitone anaesthesia (45 mg/kg) stainless steel electrodes were implanted in hippocampus (3.8 mm posterior to bregma, 2.3 mm lateral to midline and 4.5 mm ventral to the surface of the skull) and neocortex (2 mm posterior to bregma, 2 mm lateral to midline and 1.5 mm ventral to the surface of the skull) (Paxinos and Watson, 1982). A polyethylene cannula (2 mm posterior to bregma,
2 mm lateral to midline and 4 mm ventral to surface of skull) for intracerebroventricular (icv) administration of DA was also implanted with dental cement taking all necessary aseptic precautions. The animals were housed individually in cages for three days to provide for recovery from surgical trauma. Each animal was acclimatized to recording room for 24 hr preceding the experiment. EEG recordings were carried out by connecting the electrodes by means of a cable to a Grass Model 7D Polygraph (Grass Instruments Co., Quincy, MA, USA), permitting free movement of the rat within the home cage. The direct EEG signals were recorded on a chart paper (paper speed 5-10 mm/sec) with the help of a pen oscillograph. A timer providing pips of 1 sec, 5 sec and 1 min was also used. Prior to recording EEG, calibration of EEG signals for amplitude was done by determining the height of the signal having voltage of 50 μV. Also, frequency was obtained by calculating the number of cycles or peaks per sec. The changes in EEG were recorded simultaneously for cortex and hippocampus before and after drug administration. EEG changes were also recorded immediately after icv administration of DA. Each animal served as its own control and mean % change in amplitude (microvolts; μV) or frequency (cycles per sec; CPS) was calculated from EEG recorded prior to drug administration.
Drug administration

B-HT 920 (0.1-0.5 mg/kg), SKF 38393 (5 mg/kg) or rimcazole (6 mg/kg) was administered 30 min prior to the recording of EEG. Apomorphine (0.5 mg/kg) was given 15 min prior to recording EEG while recording was done immediately after icv administration of DA (10 µg/rat). Haloperidol (0.5 mg/kg) or idazoxan (1 mg/kg) was administered 15 min prior to the administration of agonists, respectively. All the drugs except dopamine were administered i.p. in a constant volume of 0.5 ml/100 g of body weight. The selection of doses was based on previous reports from our laboratory. For icv administration chronic implantation of polyethylene cannula was done as reported by Noble et al. (1967). A dose of 10 µg in 20 µL was slowly administered using a Hamilton microsyringe. Each group comprised 5 rats.

MODULATION OF MK 801 RESPONSE BY DOPAMINERGIC AGENTS IN MICE

Measurement of stereotypy

Mice were placed individually in glass containers (500 cc capacity). Sniffing, rearing, licking, biting and grooming were observed as stereotypic behaviours at 0, 5, 10, 15, 30, 45, 60, 90 and 120 min after drug administration. Mice were observed for at least 2 min and the intensity of stereotypy was measured as described by Costall and Naylor (1973). The cumulative stereotypy score was calculated by adding all scores for the purpose of comparison (Verma and Kulkarni, 1991a).
Drug administration

Various doses of MK 801 (0.1-0.5 mg/kg) and ketamine (2.5-10 mg/kg) were administered in naive mice. Apomorphine (0.1-0.5 mg/kg) was administered 15 min after the administration of MK 801. Ketamine (2.5 mg/kg) was administered at the same time as apomorphine. Haloperidol (0.5 mg/kg), molindone (2.5 mg/kg) or clozapine (7.5 mg/kg) was administered 30 min prior to the administration of MK 801 except SCH 23390 (0.1 mg/kg), which was administered 15 min prior to the administration of MK 801 and its combination with SKF 38393, respectively. SKF 38393 (5 mg/kg) or B-HT 920 (0.25 mg/kg) was given 15 min prior to the administration of MK 801. Reserpine (5 mg/kg) was given 24 hr while AMPT (150 mg/kg) was injected 1 hr prior to the experiment. Corresponding vehicles were used for all control injections. All drugs were freshly prepared prior to experimental sessions and administered i.p. in a constant volume of 1 ml/100 g of body weight. The selection of doses was based on the earlier reports from our laboratory. Each group comprised 5-18 mice.

D<sub>1</sub>/D<sub>2</sub> DOPAMINE AND N-METHYL-D-ASPARTATE (NMDA) RECEPTOR PARTICIPATION IN EXPERIMENTAL CATALEPSY IN RATS

Measurement of Catalepsy

The method is essentially the same as that of Morpurgo (1962). Various stages of catalepsy were induced
in rats with perphenazine (5 mg/kg), haloperidol (2 mg/kg)
and SCH 23390 (1 mg/kg), either alone or in combination. The
development and severity of the four stages of catalepsy
were scored according to Kukarni et al. (1980) as follows:
stage 1: rat moves freely when placed on the table,
score=0; stage 2: rat moves only when touched or pushed,
score=0.5; stage 3: rat fails to correct posture in 10 sec
when front paws are placed alternately on a 3 cm high block,
score=0.5 for each paw with a total score of 1.0 for this
stage; stage 4: rat fails to correct posture in 10 sec when
the front paws are placed alternately on a 9 cm high block,
score 1.0 for each paw, with a total score of 2.0 for this
stage. Thus, a complete cataleptic response was described
when the score was 3.5. A lower score meant an apparently
lesser degree of catatonia. The scoring of catalepsy
response was done at 5,10,15,30,45,60,90,120,180 and 240 min
after the administration of cataleptogenic agent(s).

Drug Administration

B-HT 920 (0.05-0.25 mg/kg), SKF 38393 (5 and 10
mg/kg), MK 801 (0.025-0.5 mg/kg), scopolamine (0.5 mg/kg) or
clonidine (0.05 mg/kg) was administered 5 min prior to the
administration of perphenazine (5 mg/kg), haloperidol
(2 mg/kg) or SCH 23390 (1 mg/kg). Bromocriptine (1 mg/kg)
was administered 2 hr prior to perphenazine. Corresponding
vehicles were used for all the control injections. All the
drugs were freshly prepared prior to experimental sessions.
and administered i.p. in a constant volume of 0.5 ml/100 g body weight. The selection of doses was based on the earlier reports from our laboratory. Each group comprised n=5-18 rats.

MODULATORY ROLE OF D$_1$ AND D$_2$ DOPAMINE RECEPTOR SUBTYPES IN NOCICEPTION IN MICE

Measurement of nociceptive threshold

The nociceptive threshold was determined as the tail-flick latencies elicited in response to noxious radiant heat (D’Armour and Smith, 1941; Kulkarni, 1980). Baseline latencies to tail-flick withdrawal from the radiant heat source (3-4 sec) were established. A cut off time of 10 sec was observed to prevent injury to the tail. A minimum of 3 trials, at 2 min interval, were recorded from each animal before the test.

Drug administration

Reserpine (2 mg/kg) was administered 4 hr prior while bromocriptine was administered 2 hr prior to the experiment. Morphine was administered 1 hr while naloxone (20 mg/kg) was administered 15 min before the experiment. B-HT 920 (0.1-1 mg/kg) or SKF 38393 (5 mg/kg) was administered 30 min prior to the test either alone or in combination. Apomorphine (0.25-1 mg/kg) was injected 15 min before the measurement of tail-flick latency. The antagonists namely haloperidol (0.5 mg/kg), sulpiride
(100 mg/kg) and idazoxan (1 mg/kg), respectively were administered 30 min prior to the agonists. All the drugs were administered i.p. in a constant volume of 1 ml/100 g of body weight. The selection of doses was based on previous reports from our laboratory. Each group comprised 5-10 mice.

**ROLE OF D₁/D₂ DOPAMINE AND N-METHYL-D-ASPARTATE (NMDA) RECEPTORS IN MORPHINE TOLERANCE AND DEPENDENCE IN MICE**

**Measurement of analgesic response**

Analgesic response was assessed by measuring tail-flick latency to radiant heat as described by D'Armour and Smith (1941) and modified by Kulkarni (1980). Baseline latencies to tail-flick withdrawal from the radiant source (3-4 sec) were established. A cut-off time of 10 sec was observed to prevent any injury to the tail. A minimum of three trials were recorded for each animal.

**Treatment schedule**

In acute studies, mice received saline followed 30 min later by morphine (10 mg/kg) or saline. In treatment groups, administration of DA agonists or MK 801, either alone or in combination was followed 30 min later by the administration of morphine (5 and 10 mg/kg). Apomorphine was, however, administered 15 min prior to morphine administration. In combination studies, apomorphine was administered 15 min after MK 801. Tail-flick latency was measured 1 hr following morphine administration.
For the induction of tolerance to morphine, mice received morphine (10 mg/kg) twice daily (9.00 and 17.00 hrs) for 9 days. On days 1, 3 and 9 the analgesic response was assessed by the tail-flick test 60 min after morphine injection (Kulkarni and Verma, 1992). In chronic studies, mice received DA agonists or MK 801, either alone or in combination, twice daily followed 30 min later by morphine (10 mg/kg) injection for 9 days. Apomorphine was similarly administered 15 min after MK 801 injection. Control experiments were performed on day 10 in the same group of animals to determine whether DA agonists or MK 801 affected the development of tolerance or whether they simply altered the behavioural expression of the analgesic response. On this day, the treatments were reversed so that the animals that had been treated with DA agonists or MK 801 followed by morphine on days 1 through 9 were challenged with saline followed by morphine, and animals that had been treated with saline followed by morphine on days 1 through 9 were challenged with the respective drug(s) followed by morphine. In addition, the animals that had received chronic treatment with DA agonists, MK 801 or their combination followed by saline for 9 days were challenged with the respective drug followed by morphine on day 10. Immediately after the tail-flick test on day 10, mice were injected with naloxone (2 mg/kg) to precipitate morphine withdrawal. The withdrawal
syndrome was assessed by placing each mouse in a 45 cm high plexiglass box and recording the incidence of escape jumps for 15 min.

All drug solutions were freshly prepared prior to experimental sessions and administered i.p. in a constant volume of 1 ml/100 g of body weight. The control animals received the equivalent volume of vehicle. Each group comprised 8-15 mice.

**EFFECT OF D₁/D₂ DOPAMINE RECEPTOR AGONISTS AND MK 801 ON DOPAMINE CONTENT OF WHOLE BRAIN OF RAT**

**Assay for DA levels**

The rats were sacrificed by decapitation. Whole brain was removed and homogenized in butanol for the estimation of DA. The levels of DA in whole brain were estimated spectrophotofluorometrically (Hitachi 650-40) by the method of Cox and Perhach (1973).

**Materials**

Analytical grade reagents and glass distilled water were used.

**Standard**

Dopamine hydrochloride was prepared with 0.01 N hydrochloric acid (HCl; BDH).

**Acidified butanol**

Acidified butanol was prepared by adding 0.85 ml of concentrated HCl to one litre of n-butyl alcohol.
0.1 M EDTA-1 M sodium acetate (pH 7)

0.1 M EDTA (disodium ethylenediamine tetra acetate dehydrate) (37.2 g) was dissolved in 1 M sodium acetate and made up to a volume of 1 litre. The pH was adjusted to 6.7-7 by addition of sodium hydroxide.

0.1 N Iodine

1.27 g of iodine was dissolved in 100 ml of absolute ethanol.

Alkaline sulfite

One ml of sodium sulfite solution (2.5 g of anhydrous salt in 10 ml of water) was diluted with 9 ml of 5 N sodium hydroxide just before experimental use.

Alumina

About 200 g of chromatographic grade alumina (BDH) was boiled in 1 litre of 1 N HCl for 30 min and washed with 20x100 ml of water or until the pH of the washing had risen to between 4 and 5. Finally, it was left to dry overnight at room temperature and then heated at 200°C for 2 hr.

Assay procedure

Brain samples were homogenized in ice cold acidified butanol with a mechanical mixer (Silverson Machines Ltd., UK), the volume of butanol being ten times the weight of the whole brain. The homogenates were centrifuged for 5 min at 800 g. A 2.5 ml portion of the supernatant fluid was transferred to a test tube containing 2.5 ml of distilled water and 5 ml of heptane. The tubes
were shaken for 5 min and centrifuged at 800 g for 5 min. 2.5 ml of the aqueous phase was transferred to a tube containing 200 mg of alumina. 1 ml of 2 M sodium acetate was added and the tubes were shaken for 10 min and centrifuged for 5 min at 800 g. The alumina was then washed by shaking with 2 ml of distilled water for 5 min and centrifuged for 5 min at 800 g. The aqueous phase was discarded and 2 ml of 0.1 N acetic acid was added to alumina. The tubes were gently shaken for 10 min and centrifuged at 800 g for 5 min; 1 ml of aqueous phase was transferred to a small test tube (13x75 mm) for fluorescent assay of DA.

For the estimation of DA, the pH of the acetic acid extract was adjusted to 4. To this solution 0.2 ml of 0.1 N EDTA reagent was added and the mixture was adjusted to a pH of 6.5. Then, 0.1 ml of 0.1 N iodine was added to oxidize the monoamine. After exactly 2 min, the oxidation was stopped by addition of 0.2 ml of alkaline sulfite and exactly 2 min later the pH of the solution was adjusted to 5.4 by addition of 0.2 ml of 5N acetic acid. To assay DA, the solution was heated at a temperature of boiling water for 5 min and the fluorescence was read at activation 320 nm and emission 370 nm.
Reagent blank

A reagent blank was carried out with the same procedure except using 2.5 ml of acidified butanol in place of tissue homogenate.

For calculation, tissue blank was subtracted from the standard and reagent blank was subtracted from the test samples. For finding out the concentration in test samples, standard curve was plotted with 250, 500, 750 and 1000 ng of DA. The concentration of test samples was calculated by linear regression analysis.

Drug administration

Various doses of B-HT 920 (0.1-0.5 mg/kg) and MK 801 (0.1-0.5 mg/kg) were administered 30 min prior to sacrificing rats by decapitation. SKF 38393 (5 mg/kg) was also given 30 min prior to sacrificing rats. In combination studies, B-HT 920 or SKF 38393 was injected at the same time as MK 801. SCH 23390 (1 mg/kg) was administered 15 min prior to the administration of the combination of SKF 38393 and MK 801. Corresponding vehicles were used for all the control injections. All the drug solutions were freshly prepared prior to experimental sessions and injected i.p. in a constant volume of 0.5 ml/100 g of body weight. Each group comprised 5-6 rats.
Fluorometric Reading

Fig. 13. Standard curve of dopamine

Concentration of Dopamine (ng/2.5ml)