COX inhibition: catalepsy and Striatum Dopaminergic-GABAergic-Glutamatergic neurotransmission

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Abstract:

Selective COX-2 and COX-1 inhibitors were administered (i.p. acutely) to normal and parkinsonian rats, followed by the analysis of the striatal dopamine, GABA and glutamate concentrations using the microdialysis technique, simultaneously, the catalepsy of animals was evaluated. Selective COX-2 inhibition showed improving effects on the catalepsy followed by decreasing the striatum glutamatergic-GABAergic and enhancing the dopaminergic neurotransmission. In addition histological studies showed that COX inhibition had not any improving effects on damaged SNc neurons.

Findings*:*

Cyclooxygenase (COX) is the first enzyme in the prostaglandin/ prostacyclin/thromboxane pathway. Three COX isoforms, COX- 1, COX-2 and COX-3 have been identified. COX-1 is the constitutive form of COX and performs a housekeeping function to synthesize prostaglandins, which are involved regulating normal cellular activities. COX-2 appears to be expressed in dendrites and cell bodies of neurons in several areas of the brain such as nigrostriatal pathway [1,2]. Prostaglandins have modulatory effects on neural transmission, especially prostaglandin E2 [the main metabolite of COX-2] PD is a neurodegenerative disease in the nigrostriatal pathway of humans, and the resultant losses of nerve terminals accompanied by dopamine deficiency in this pathway are responsible for most of the movement disorders[4].

In different paradigms, inflammation contributes to the neuronal damage and anti inflammatory agents such as dexamethasone and celecoxib have been shown to provide some neuroprotection in PD models[5,6]. The study of striatal dopaminergic-glutamatergic-GABAergic interactions has special importance due to the physiological and pathophysiological processes of these systems, such as Parkinson's disease[7]. Previously we showed that anti inflammatory agents such as COX-2 inhibitors[8] or COX-2 gene expression inhibitors[9] can improve the rigidity of parkinsonian rats with uncertain mechanisms. Investigation of the effects of COX inhibitors on catalepsy, striatum neurotransmission changes [~simultaneously] and damaged substantia nigra pars compacta (SNc) neurons recovery were interest of the study.

These animal experiments were carried out in accordance with the recommendations from the declaration of Helsinki and the internationally accepted principles in the use of experimental animals.

Each rat was anesthetized separately by injection of 75 mg/kg ketamine combined with 8 mg/kg Xylazin i.p. Then the rats were placed in the stereotaxic instrument. The left SNc, region of the nigrostriatum was targeted at the following coordinates: -4.8 mm posterior and -1.6 mm lateral to bregma and 8.2 mm ventral to the surface of the skull for the left SNc according to the atlas[10] Vehicle (0.2% ascorbic acid) or 6-OHDA (8 μg in 1 μl in 0.2% ascorbic acid) was infused unilaterally through a 26-gauge stainless steel cannula into the SNc region thus creating hemiparkinsonian rats. Then the skull was exposed and a hole was drilled through it in the area overlying the right striatum, using the following coordinates with respect to the bregma: A/P + 1 mm; M/L + 3 mm, D/V + 6 mm according to the atlas[10]. A guide-cannula lowered into the brain was fixed to the cranium and the incision was closed. Rats were allowed to recover from the surgery for 7–10 days.

Microdialysis experiments were performed in normal and SNc-lesioned animals. On the experimental day a microdialysis probe was inserted into the cannula, and the inputs of the probes

were connected to a microperfusion pump CMA/102 infusion pump (CMA/Microdialysis, Sweden), which delivered a modified Ringer solution (147 mM NaCl, 1.2 mM $CaCl_2$, 2.7 mM KCl, 1.0 mM $MgCl_2$ and 0.04 mM ascorbic acid) through the probe at a flow rate of 2 µl/min. Ringer solution was then infused for 3–3.5 h before the baseline samples were collected to obtain stable basal extracellular levels of dopamine, glutamate or GABA. The microdialysate samples (20 µl) were collected every 20 min. When a stable outflow was shown by four consecutive samples of neurotransmitters, rats were given Celecoxib (3, 6 mg/kg) and Compound 11_b (2, 4 mg/kg) that we previously reported it[11] as a new selective COX-2 inhibitor and SC-560 (1, 2 mg/kg) and DMSO as their vehicle. Control rats received a saline injection (1 ml/kg) i.p. The dialysates were collected for 4 h after the administration of COX-2 or COX-1 selective inhibitors. The stress caused by the i.p. vehicle injection, catalepsy evaluation and handling of the rats was not found to alter the extracellular glutamate-dopamine levels. In part of experiments, when the rats were given drugs or vehicle after four stable consecutive samples, the dialysates were collected for 2.5h after the injection. Microdialysate levels of glutamate and dopamine were analyzed immediately. Dopamine was analyzed by reverse-phase HPLC with electrochemical detection[12] and the HPLC with flourometric detection was used for determination of glutamate-GABA[13].

The electrochemical detection (ECD) system consisted of a shimadzu SCL 10-Avp system controller, LC-10AS pump, SIL-10A cooled autoinjector, CT0-10A oven, LECD-6A electrochemical detector and a Gastorr online degasser (ISS, England). System components were used in conjunction with Class VP-5 software (Shimadzu). All samples were injected onto a reversed phase Luna 5 µ C18 250 mm × 4.6 mm column (Phenomenex), which was protected by Krudkatcher disposable pre-column filters (Phenomenex) and SecurityGuard cartridges (Phenomenex).

The fluoresecent detection (FLD) system consisted of a Waters 510 pump; 717plus cooled Autosampler, a Hewlett Packard 1046A fluorescent Detector, a waters busSAT/IN module and a Croco-Cil column oven. System components were used in conjunction with Waters Empower software. All samples were injected onto a reversed phase Luna 3 µ C18(2) 150 mm × 2 mm column (Phenomenex), which was protected by Krudkatcher disposable pre-column filters (Phenomenex) and SecurityGuard cartridges (Phenomenex).

The mobile phase that was used on the ECD system was composed of a mixture of 0.1M di-sodium hydrogen orthophosphate/50 µM EDTA (pH 5.6, 1 M OPA) and HPLC grade methanol (35:65). Mobile phase was filtered through Millipore 0.45 µm HV Durapore membrane filters (AGB, Dublin) and vacuum degassed prior to use. Compounds were eluted isocratically over a 20 min runtime at a flow rate of 0.65 ml/min after a 20 µl injection. The column was maintained at a temperature of 30 °C and samples/standards were kept at 4 °C in the cooled autoinjector prior to analysis unless otherwise stated. The glassy carbon working electrode combined with an Ag/AgCl reference electrode (Shimadzu) was operated at +0.8 V unless otherwise specified and the range of the detector was set to 1.

The mobile phase which was used on the FLD system was composed of a mixture of 0.1 M di-sodium hydrogen orthophosphate/50 µM EDTA (pH 5.3, 1 M OPA) and HPLC grade methanol (35:65). Mobile phase was filtered through Millipore 0.45 µm HV Durapore membrane filters (AGB, Dublin) and vacuum degassed prior to use. Compounds were eluted isocratically over a 15 min runtime at a flow rate of 0.1 ml/min after a 20 µl injection. The column was maintained at a temperature of 30 °C and samples/standards were kept at 4 °C in the cooled autoinjector prior to analysis unless otherwise stated. The fluorescent detector was set at an excitation wavelength of 420 nm, an emission wavelength of 480 nm and a PMT gain of 5.

Simultaneously to microdialysis experiments catatonia was assessed at 30 minute (min) intervals until 120 min and at the end of 240 min by means of standard bar test[14,15]. Unilateral lesion of SNc was chosen so that it could elicit a moderate degree of catalepsy and thus enable the detection of either attenuation or potentiation of phenomenon[8,9] . Catalepsy was assessed in terms of the times for which the rat maintained an imposed position with both front limbs extended and resting on a 10 cm high wooden bar (1.25 cm diameter). The end point of catalepsy was considered to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. A cut-off time of 300 seconds was applied. In the study, the drugs were administered only once at time 0 min. After the experiments, rrecovery of damaged SNc neurons was evaluated using the histological studies by preparation of 50 µm coronal brain sections as previously described[9].

The data was shown as the mean ± SEM and the catalepsy-data was analyzed by one way ANOVA followed by Dunnet Multiple Comparision test and for microdialysis data statistical evaluation of the results was performed by means of ANOVA and Student–Newman–Keuls multiple range test, considering the following significant differences: $P < 0.05$. The average concentration of four stable samples before drugs or vehicle [DMSO] administration was considered as the basal GABA-glutamate-dopamine concentrations. In the study, the vehicle and all doses of the test drugs 30 min after the last microdialysis sampling for baseline determination were administered. However, from 60 min onwards after the administration, celecoxib (3, 6 mg/kg) and compound11_b (2, 4 and 8 mg/kg) resulted in significantly ($P<0.05$) lower cataleptic scores than the vehicle-treated mice. The results of the study showed that the high dose of Compound11b was as protective as the 6 mg/kg dose of that of celecoxib. Our statistical analysis showed that the protective effect of compound 11b against SNc-lesion-induced-catatonia was both dose-and time-dependent. In addition no significant changes in the catalepsy have been observed after selective COX-1 inhibitor SC560 administration. Moreover, statistically neither

selective COX-1 nor COX-2 inhibitors were able to change the muscular movement of normal rats. Catalepsy scores of investigated groups [each group contained 6 rats] were depicted in Tables1, 2 as Mean ± SEM. The striatal extraneuronal (i.e. microdialysate) concentration of glutamate in normal and SNc-lesioned rats of all examined groups before drug-vehicle injection (baseline) were 2.3±0.25 and 2.82±0.35 ng/20µl, and dopamine concentrations of normal and SNc lesioned rats were 3.16±0.51 and 2.1±0.35 pg/20µl and for GABA 0.129±0.02 ng/20µl [normal] and 0.391±0.06 ng/20µl [SNc lesioned] respectively.

COX-2 inhibitors and their doses were observed to modify GABAergic-glutamatergic-dopaminergic neurotransmission in the striatum of hemiparkinsonian rats during within the observation period, as depicted in Fig. 1_{A-F}. Additionally the changes were seen to be significant ($p<0.05$) for GABA-glutamate-dopamine concentrations on or after 40 min throughout until 180 min. Statistically only administration the high doses of COX-2 inhibitors to normal rats had the significant decrease in glutamatergic and increase in dopaminergic neurotransmission $P<0.05$ at 60-120 min only. Furthermore SC-560 had no significant effect on GABAergic-glutamatergic-dopaminergic neurotransmission in normal and SNc lesioned rats [Figs. 2_{A-F}]. Interestingly all doses of COX-2 inhibitors affected the GABA neurotransmission in the straitum of normal rats. Histological results showed that acute treatment with either COX-2 or COX-1 inhibitors did not improve the lesion at all [Fig3_{A-D}]. This study was intended to explore the more important role for COX-2 but not for COX-1 in glutamatergic-dopaminergic neurotransmission in striatum or rigidity improvement in PD model. Furthermore this study shows that acute COX inhibition had no improving effect on damaged SNc neurons. According to the results selective COX-2 inhibition affects on normal striatal neurotransmission but not induced movement abnormalities.

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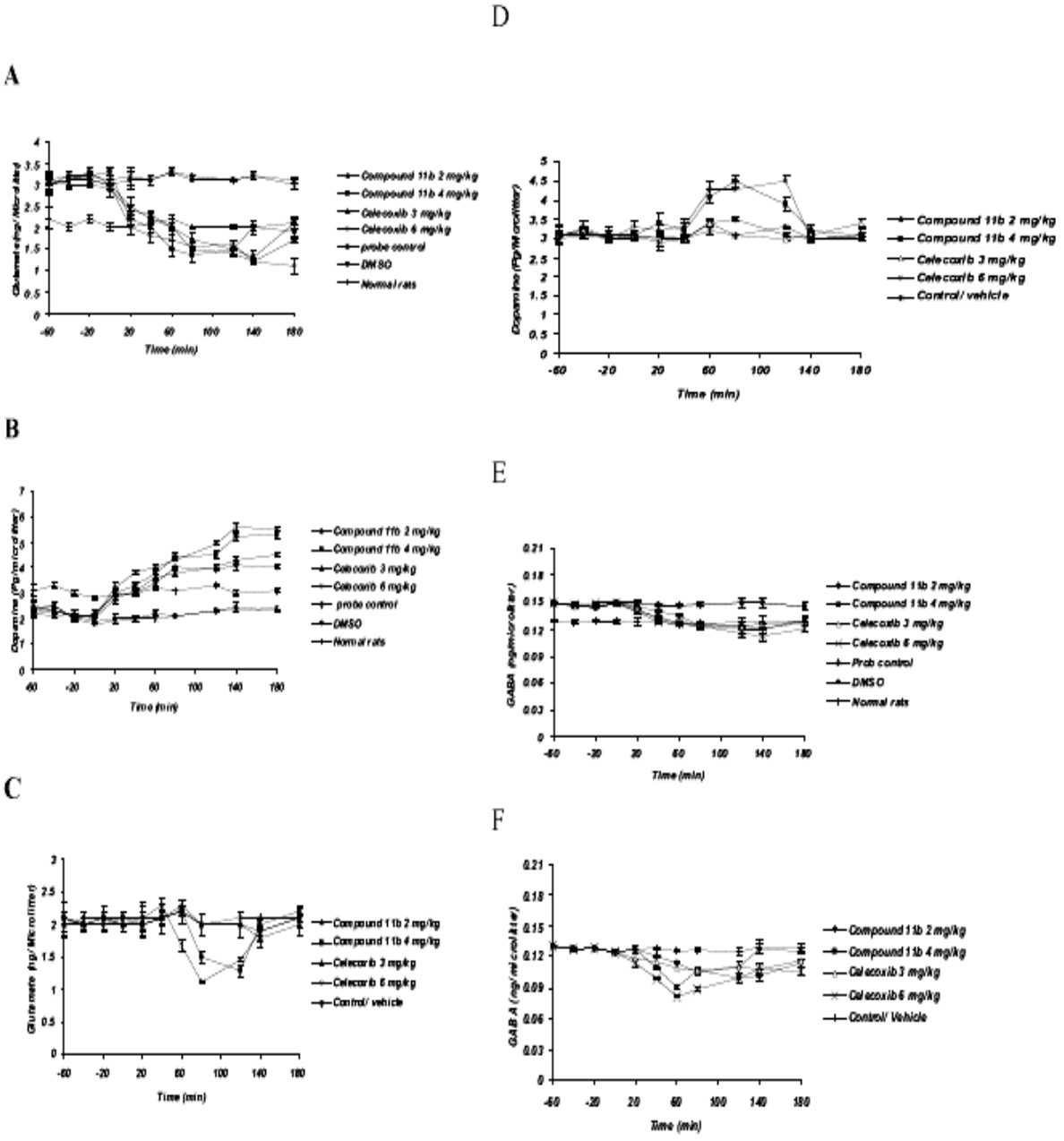

Fig. 1 *COX-2 inhibitors can significantly P<0.05 decrease the Striatum glutamatergic-[A/C] GABAergic [E/F] neurotransmission and enhance the Striatum dopaminergic neurotransmission [B/D] in normal and SNc lesioned rats.*

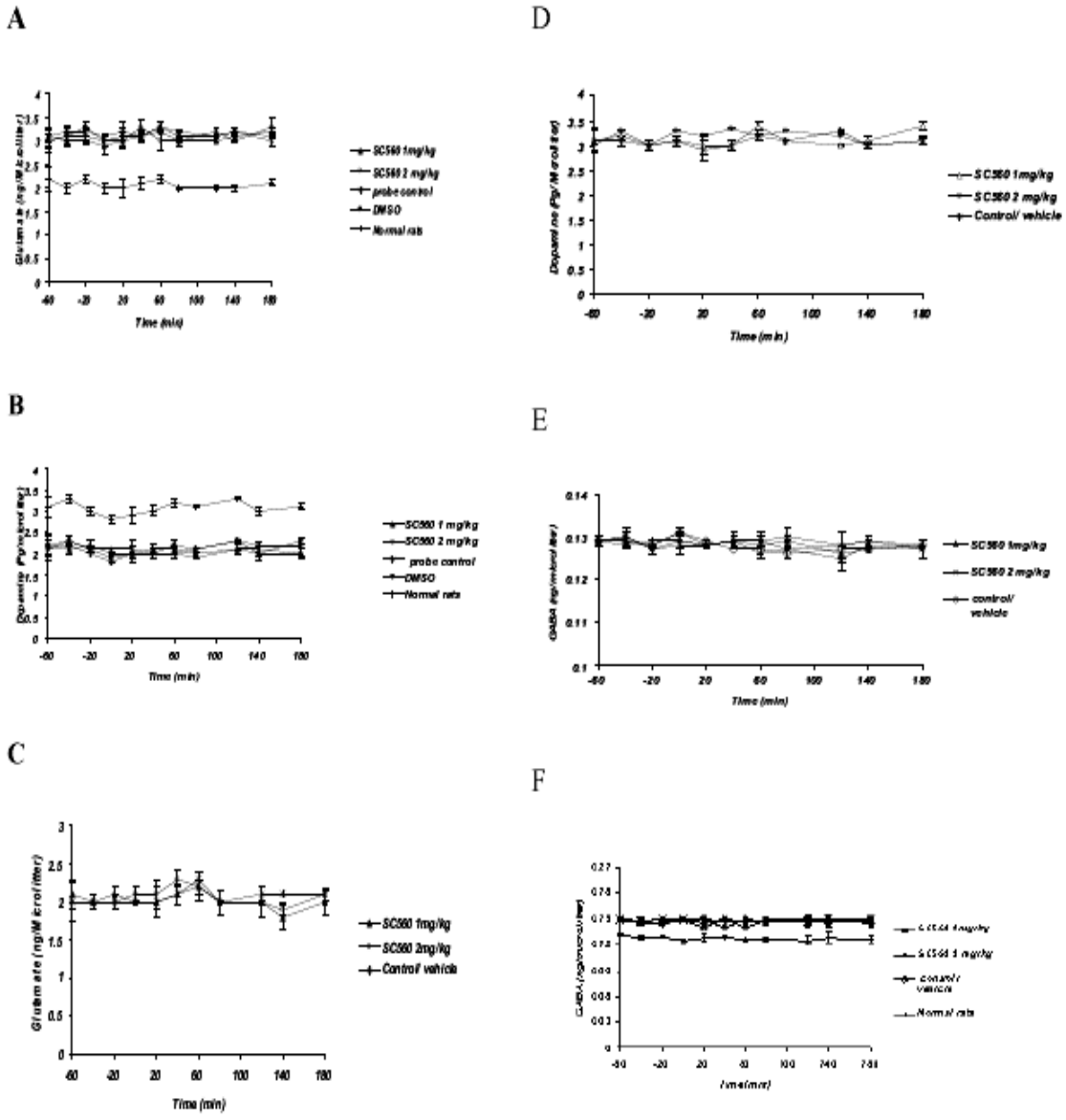

Fig. 2 ***COX-1 inhibitions can not significantly P<0.05 modify the Striatum glutamatergic-[A/C] GABAergic [E/F] neurotransmission or the Striatum dopaminergic neurotransmission [B/D] in normal and SNc lesioned rats.***

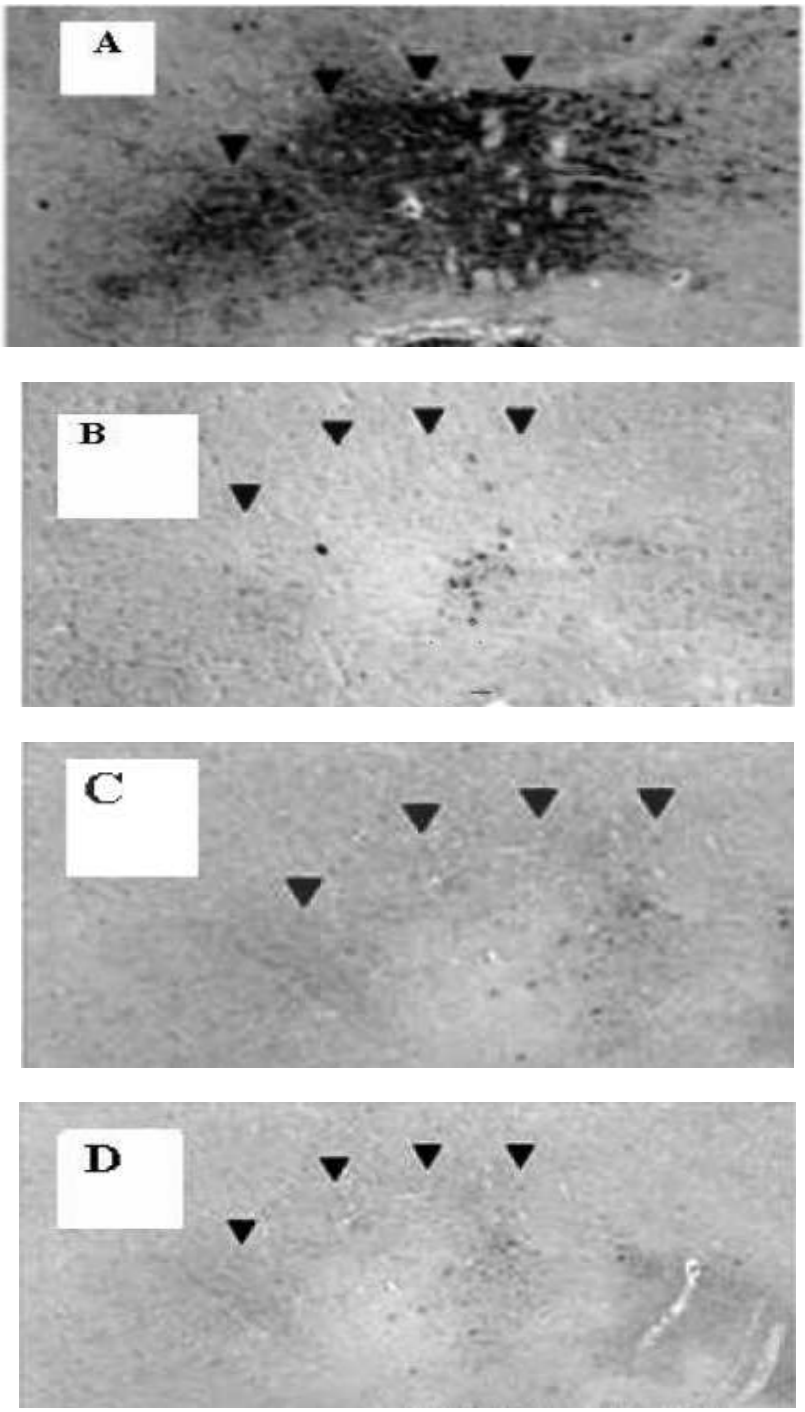

Fig. 3 : ***A***: *SNc with 4X magnification under the light microscope in normal rats.* ***B***: *Lesioned SNc with 4X magnification before any treatment.* ***C***: *Acute selective COX-2 inhibition did not improve the dopaminiergic Lesion.* ***D:*** *Acute selective COX-1 inhibition did not improve the dopaminiergic Lesion.*

Table 1*: The catalepsy scores [Mean±SEM] in parkinsonian rats after drug-vehicle administration.*

Groups/ Time after administration (min)	*30*	*60*	*90*	*120*	*240*
Control/Vehicle	*49.50± 2.16*	*51.33±2.43*	*50.24±3.40*	*49.15±2.11*	*50.11±2.21*
Celecoxib 3 mg/kg	*40.01±1.84*	*32.21±1.16*	*26.23±1.21*	*24.31±1.00*	*26.12±0.88*
Celecoxib 6 mg/kg	*35.12±1.12*	*20.14±1.62*	*15.21±1.28*	*18.32±1.19*	*17.16±1.74*
Compound11$_b$ 2 mg/kg	*44.11±2.21*	*28.23±1.43*	*30.01±0.93*	*33.21±0.66*	*40.00±1.60*
Compound11$_b$ 4 mg/kg	*38.09±1.00*	*20.27±0.78*	*18.22±1.11*	*24.03±1.43*	*29.12±1.32*
SC560 1 mg/kg	*50.50± 2.16*	*50.33±2.43*	*49.74±2.23*	*51.15±2.41*	*50.01±1.21*
SC560 2 mg/kg	*48.90± 1.43*	*50.40±1.17*	*51.26±2.33*	*50.15±1.33*	*51.09±0.91*

Table 2: *The catalepsy scores [Mean± SEM] in normal rats after drug-vehicle administration.*

Groups/ Time after administration (min)	*30*	*60*	*90*	*120*	*240*
Control/Vehicle	*3.98± 2.44*	*4.33±1.36*	*4.24±1.00*	*4.15±1.33*	*3.91±2.01*
Celecoxib 3 mg/kg	*4.44± 0.87*	*4.08±2.12*	*4.74±1.66*	*3.75±1.43*	*4.11±1.73*
Celecoxib 6 mg/kg	*4.01± 1.14*	*3.53±0.89*	*4.24±1.08*	*4.45±1.56*	*3.89±1..01*
Compound11$_b$ 2 mg/kg	*3.90± 2.03*	*4.11±1.36*	*4.04±1.22*	*4.23±1.41*	*3.93±2.14*
Compound11$_b$ 4 mg/kg	*4.17± 1.13*	*4..09±1.04*	*4.29±1.43*	*3.93±1.01*	*4.00±1.63*
SC560 1 mg/kg	*4.19± 0.97*	*3.99±1.47*	*3.96±1.22*	*4.35±1.03*	*3.97±1.15*
SC560 2 mg/kg	*3.88± 1.33*	*3.88±1.11*	*4.34±1.00*	*4.49±0.65*	*4.01±1.39*