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Biochemical and Pharmacological Evidence for Central Cholinergic Regulation of Shock-Induced Aggression in Rats

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RAY, A., P. SEN AND M. ALKONDON. *Biochemical and pharmacological evidence for central cholinergic regulation of shock-induced aggression in rats.* PHARMACOL BIOCHEM BEHAV **32**(4) 867–871, 1989.—Acetylcholinesterase (AChE) activity was estimated in brain and heart homogenates and plasma of 'aggressive' and 'nonaggressive' rats. Brain homogenates of 'nonaggressive' rats hydrolyzed significantly more substrate when compared to the 'aggressive' rats. Such differences were not seen in the heart homogenates or plasma of these two groups of rats. Acute DFP (0.1, 0.3 and 1.0 mg/kg) attenuated shock-induced aggression (SIA) 2 hr after treatment but facilitated SIA 24 hr and 48 hr after drug administration. Long-term DFP (0.3 mg/kg × 10 days), on the other hand, induced a significant enhancement in the SIA score, whereas atropine (1.0 and 5.0 mg/kg) produced a dose-related attenuation of the same. Pretreatment of rats with atropine (5 mg/kg) antagonized the long-term DFP-induced facilitation of SIA. These results are discussed in the light of an inhibitory central cholinergic mechanism in the regulation of SIA.

Acetylcholinesterase Aggressive Nonaggressive DFP Atropine Shock-induced aggression

SHOCK-INDUCED aggression (SIA) is one of the reliable and effective models of experimental (defensive) aggression in rodents. Complex neurochemical mechanisms may explain the regulation of SIA and brain biogenic amines are importantly involved (9, 19, 23, 31–34, 42). Intense autonomic activation is apparently associated with SIA and adrenergic-cholinergic interactions in the modulation of this phenomenon is reported (23, 34, 42). The role of the cholinergic system is, however, less clear. Some studies have indicated facilitatory cholinergic mechanisms in SIA, but an inhibitory role for cholinergic transmission has also been suggested (34,37).

Rats exhibited characteristically different behavioral patterns when exposed to footshock in pairs (31–34). For example, some rats adopted dominantly aggressive posture while others remained submissive and showed no desire to ward off or challenge the aggressor. Accordingly, the former group was called 'aggressive' and the latter 'nonaggressive.' When two 'aggressive' rats are shocked in pairs, a typical fighting response is elicited.

The purpose of this study was to assess possible central cholinergic regulatory mechanisms which determined the aggressivity of the rats. The presence of transmitter synthesizing and/or degrading enzymes are important determinants of synaptic transmission (35). Acetylcholinesterase (AChE) is used as a marker of central cholinergic neurons (4, 15, 18, 24–26, 38, 39). Further, pharmacological modulation of AChE enzyme activity in the brain is also used to explain changes in central cholinergic neuroregulatory mechanisms (5, 6, 8, 14, 28, 34, 40, 41, 45). Thus, the brain AChE enzyme activity was estimated in the 'aggressive' and 'nonaggressive' rats. We also evaluated the effects of acute and long-term administrations of the AChE inhibitor, diisopropylfluorophosphate (DFP) on SIA.

METHOD

Subjects and Screening

Male Wistar rats (200–250 g) were housed in light (12 hr light:12 hr dark schedule) and temperature (22 ± 2°C) controlled conditions. They had free access to food and water until the morning of the day of the experiment. For screening, randomly

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paired rats were exposed to footshock for 1 min in a shock chamber (Aggressometer, Techno Electronics). This chamber is a ventilated, perspex box (21 × 17 × 15 cm) with a parallel grid floor, through which alternate current could be delivered by an inbuilt shock generator. The stimulation parameters were 100 V, 0.5 mA, 5 Hz and 5 msec pulse duration. Some rats exhibited typical fighting postures (31–34) and were screened as 'aggressive' when footshock was given. Other rats remained submissive and showed no desire to ward off or challenge the aggressor. These animals were labelled 'nonaggressive.'

Estimation of AChE Enzyme Activity

Both aggressive and nonaggressive rats were used for AChE enzyme estimations. Animals were killed by cervical dislocation and the blood was collected by cardiac puncture. The brains (exclusive of cerebellum) and hearts were quickly removed, washed with saline and minced and homogenized in cold phosphate buffer (pH 8.0). The blood was centrifuged to obtain the plasma. The brain and heart homogenates were centrifuged at 10,000 rpm at 0°C for 10 min. The supernatents were tested for AChE enzyme activity, which was determined photometrically (30). The protein contents were also estimated by the method of Lowry *et al.* (27) and the enzyme activity was expressed as the number of nanomoles (nmols) of substrate hydrolyzed per mg protein per min. Methacholine iodide was used as substrate for brain tissue (2), whereas acetylcholine was used for the heart and the plasma. The AChE enzyme activities of the brain, heart and plasma of 'aggressive' and 'nonaggressive' rats were compared using the Mann-Whitney U-test (two-tailed).

Evaluation of SIA

Rats were housed and screened in a manner similar to those described earlier and the 'aggressive' rats were selected for this study. On exposure to footshock in pairs, the rats exhibited a typical fighting episode. They stood up to their hindlimbs and struck at (with forepaws), wrestled with or bit at each other and the scoring was as follows: one strike was scored as 1, one wrestling bout as 2, one bite without bleeding as 3 and one bite with bleeding as 4. Each pair of rats were footshocked at a time for 3 min each, before as well as after drug or vehicle administrations and the cumulative score during each 3 min period of electroshock was counted with a digital counter. The differences (predrug~postdrug) in the aggressive scores were calculated for each pair in all the treatment conditions. This method of scoring was found to be very objective and used effectively in some of our earlier studies (31–34).

In the initial study, vehicle or DFP (0.1, 0.3 and 1.0 mg/kg, IP) was administered acutely and the same rats were shocked in pairs at 2 hr, 24 hr, and 48 hr, respectively, after a single drug treatment. The differences in the aggressive scores [predrug (0 hr)~postdrug] were calculated at 2 hr, 24 hr and 48 hr, for each pair, in all treatment groups. These differences were then analyzed using the Wilcoxon matched-pairs signed-ranks *t*-test for correlated measures.

In the next study, DFP, atropine sulfate or vehicle (saline) was injected once daily for 10 consecutive days in a chronic treatment schedule, to randomly paired rats. Twenty-four hours after the last injection the same pairs were footshocked and the aggressive scores were determined in a manner described earlier. The differences (before treatment~after treatment) in the scores were calculated for each group and subjected to statistical analysis.

Jump latencies were determined both in the acute and long-term treatment conditions, as a measure of nociception, by noting the time (sec) the rat had all four paws off the grid floor of the shock chamber (29), when footshocked under conditions used to elicit aggression.

TABLE 1

CHOLINESTERASE (ChE) ENZYME ACTIVITY IN THE BRAIN, HEART AND PLASMA OF 'AGGRESSIVE' AND 'NONAGGRESSIVE' RATS

Group of Rats	ChE Enzyme Activity (nmol of substrate* hydrolysed/mg protein/min) (mean ± S.E.) Brain	Heart	Plasma
Aggressive	12.88 ± 1.02	13.12 ± 0.82	49.82 ± 3.69
Nonaggressive	23.70 ± 1.72[a]	13.29 ± 0.81	52.72 ± 2.51

*Methacholine for brain homogenates, whereas acetylcholine for heart and plasma.

[a]$p<0.002$ compared to aggressive group (Mann-Whitney U-test, two-tailed).

n = 10, for brain homogenates and n = 6, each, for heart homogenates and plasma.

Drugs

Di-isopropylfluorophosphate (DFP) and atropine sulfate were purchased from Sigma Chemical Company (St. Louis, MO). All drugs or vehicle (saline) injections were made intraperitoneally (IP) in a volume of 1 ml/kg.

RESULTS

Biochemical Data

Analysis of the data of biochemical assay for AChE showed that the brain AChE activity was higher in 'nonaggressive' rats. As shown in Table 1, the amount of substrate hydrolyzed by the 'nonaggressive' rat brain homogenates was almost twice as much as in the 'aggressive' rats ($p<0.002$). No such differences were observed between the substrate hydrolyzing ability of the heart homogenates or plasma ($p>0.05$).

Effects of DFP on SIA

Acutely administered DFP (0.1, 0.3 and 1.0 mg/kg) produced a dose-dependent inhibition of SIA in rats tested 2 hr after drug treatment. Analysis showed that the differences between the 0 hr and 2 hr scores were significant ($p<0.05$ and $p<0.01$ respectively, Wilcoxon matched-pairs signed-rank *t*-test) (Table 2). Higher doses of DFP (3.0 mg/kg) resulted in high incidence of mortality. Inhibitory effects on SIA were seen in some pilot experiments but this dose was not included in the present study. When the same rats (pairs shocked earlier at 0 hr and 2 hr after sublethal doses of DFP) were footshocked 24 hr and 48 hr after DFP injection, a clearcut time and dose-related potentiation of SIA was apparent. As shown in Table 2, the facilitation in the aggressive scores were more at the 48 hr period than those seen 24 hr after drug administration, the effects with DFP (0.3 and 1.0 mg/kg) being most consistent ($p<0.05$ and $p<0.01$, respectively).

On the other hand, long-term DFP (0.3 mg/kg) (dose selected on the basis of earlier acute data in Table 2) had a significant potentiating effect on SIA when the rats were shocked 24 hr after the last injection of a 10-day treatment schedule ($p<0.02$, Mann-Whitney U-test, two-tailed) (Table 3). Atropine (1.0 or 5.0 mg/kg × 10 days) was seen to have an inhibitory effect on SIA—the effect with the higher dose (5 mg/kg) being statistically significant ($p=0.05$). Interestingly, when atropine (5 mg/kg) was administered chronically, 30 min prior to DFP (0.3 mg/kg), the otherwise

TABLE 2

DOSE AND TIME RELATED CHANGES IN SHOCK-INDUCED AGGRESSION AFTER ACUTE DFP ADMINISTRATION IN RATS

Treatment (mg/kg, IP)	n*	Mean Aggressive Score (± S.E.) At 0 hr	At 2 hr	At 24 hr	At 48 hr
Controls	10	86.7 ± 4.7	99.3 ± 8.1	94.5 ± 7.7	103.0 ± 9.3
DFP (0.1)	7	74.5 ± 5.0	51.5 ± 3.9[a]	101.4 ± 6.4	113.6 ± 7.8[a]
DFP (0.3)	8	89.0 ± 8.7	44.8 ± 6.4[b]	122.7 ± 10.5[a]	145.0 ± 12.5[b]
DFP (1.0)	8	98.0 ± 7.8	50.6 ± 5.6[b]	131.1 ± 12.8[a]	164.9 ± 6.9[b]

*Pairs of rats.

[a]$p<0.05$; [b]$p<0.01$, compared to respective 0 hr (pretreatment data), Wilcoxon matched-pairs signed-rank t-test.

facilitatory effect of long-term DFP on SIA was attentuated. As seen in Table 3, the aggressive score for the A + DFP group was not significantly different from those of the control group ($p>0.05$).

The jump latency data showed that only acute DFP (1.0 mg/kg) significantly altered the mean jump latencies (sec) 2 hr (and not 24 hr or 48 hr) after a single injection [pre-DFP (0 hr): 6.9 ± 0.4 vs. DFP (2 hr): 9.7 ± 1.0; n = 8; $t = 3.85$; $p<0.05$, paired t-test]. However, measurements of jump latencies for the long-term treatment groups showed that the data with DFP and atropine were not significantly different from the respective control values ($p>0.05$; data not shown).

DISCUSSION

The present results indicate involvement of a cholinergic neuroregulatory mechanism in SIA. Acetylcholinesterase (AChE) is used as a histochemical marker of central cholinergic neurons (4, 15, 18, 24–26, 38, 39) and drug-induced modulations of AChE is used to express involvement of the cholinergic system in pathophysiological processes (5, 6, 8, 14, 28, 34, 40, 41, 45). The presence and/or activity of the AChE is a good index of cholinergic function (or tone) at different target sites and the differential levels of this enzyme in the brains of 'aggressive' and 'nonaggressive' rats shows the possible significance of this enzyme and endogenous brain cholinergic activity in the regulation of SIA. 'Aggressive' rats showed lower brain AChE activity as compared to their 'nonaggressive' counterparts—the reduced enzyme activity being a probable index of increased aggressiveness. Interestingly, no significant differences were seen in the ChE enzyme activity of heart homogenates and plasma, between these two groups of rats, indicating that these biochemical differences are more important in the brain. This hypothesis is consistent with reports that atropine (and not methylatropine) reversible effects on SIA was seen with drugs which enhanced brain cholinergic activity by way of AChE inhibition (34).

TABLE 3

EFFECTS OF LONG-TERM (× 10 DAYS) TREATMENT WITH DFP AND ATROPINE ON SHOCK-INDUCED AGGRESSION IN RATS

Treatment (mg/kg, IP)	n*	Mean Aggressive Score (± S.E.) Before Drug	After Drug	Differences†
Controls	10	84.2 ± 6.0	93.3 ± 6.8	(+) 9.1 ± 5.0
DFP (0.3)	9	90.3 ± 9.5	130.0 ± 13.9	(+)39.7 ± 5.6[a]
Atropine (A, 1.0)	8	66.0 ± 7.2	45.7 ± 4.6	(−)20.3 ± 8.1
A (5.0)	8	75.8 ± 7.6	48.3 ± 5.3	(−)27.5 ± 6.6[b]
A (5.0) + DFP (0.3)	8	72.3 ± 5.8	84.2 ± 6.2	(+)11.9 ± 3.9

*Pairs of rats.

†(+) and (−) prefixes indicate increase and decrease in the score.

[a]$p<0.02$; [b]$p<0.05$ (compared to controls, Mann-Whitney U-test, two-tailed).

It could also be argued that lower AChE activity indicates enhanced ACh levels in aggressive rats (as AChE hydrolyses ACh). However, the results with the DFP show that brain AChE inhibition by this drug (17,43) in these 'aggressive' rats reduced aggressiveness. An earlier report with cholinomimetics and some related drugs had also indicated this (34). These various observations indicate that central cholinergic neurotransmission attenuates SIA. Inhibitory cholinergic mechanisms have also been suggested for other models of 'defensive' aggression (1). Earlier data had suggested a facilitatory cholinergic involvement in the modulation of SIA (37), but our present contention is apparently not in agreement with the same.

Acute and long-term administration of DFP showed differential effects on SIA. The data clearly shows a biphasic time curve for the aggression response to this anti-ChE agent. Whereas immediate acute effects (2 hr) were inhibitory, the delayed effects (24 hr and 48 hr) after a single injection, were more of a facilitatory nature. Chronic treatment, on the other hand, also tended to aggravate SIA. Long- and short-term effects of DFP in the brain have been attributed to an altered cholinergic responsiveness and pharmacological and biochemical data have supported this concept (5, 6, 8, 14, 28, 34, 40, 41, 45). An inhibitory regulation of SIA by central cholinergic neurons has already been speculated (34) and our results with acute and long-term DFP are seemingly in agreement with this hypothesis. Pain sensitivity is known to contribute importantly to SIA and its modulation by drugs (34,36). However, in the present experiments, our data with jump latencies show that nociceptive elements are apparently not essentially involved in the drug-induced changes in the SIA.

The role of cholinergic receptors in diseases of the central nervous system is a dominent theme and the plasticity of the muscarinic cholinoceptor in response to pharmacological modulation of cholinergic transmission, widely documented (5, 7–14, 20–22, 28, 40, 41, 44, 45). For example, long-term treatment with cholinomimetics down-regulates and subsensitizes muscarinic cholinoceptors. In contrast, chronic treatment with antimuscarinic agents up-regulates and supersensitizes these receptors. Long-term treatment with DFP and other AChE inhibitors reduce muscarinic binding sites in the brain (8, 14, 28, 40, 41, 45). A similar phenomenon may have occurred in our study using prolonged treatment with DFP. Reduction in cholinoceptor density is consistent with cholinergic (postsynaptic) hypofunction, which in turn could explain the enhancement in the SIA score seen with DFP (Table 3). This concept receives support from the observation that long-term atropine pretreatment also prevented the DFP-induced increase in the fighting score. As shown in Table 3, the data of the A + DFP group was not significantly different from the control values ($p>0.05$). Alternatively, long-term DFP-induced cholinergic hypofunction could have resulted in the turning on of catecholamine systems, particularly that of dopamine, the facilitatory role of which is well known in SIA (33).

Interestingly, 24 hr and 48 hr after acute (single injection) sublethal doses of DFP (0.3 and 1.0 mg/kg), there was a significant increase in the SIA score (Table 2). Short-term (acute) treatments inducing up- and down-regulation of muscarinic receptors are carefully described in the literature (3, 8, 28). Irreversible, long-acting effects of DFP on AChE is also documented. Thus, it

is possible that the delayed facilitatory effects of acute DFP on SIA may have resulted from altered physiology of the cholinergic system such as receptor down-regulation, a result similar in nature to that indicated in the long-term studies (Table 3). This effect on SIA was more pronounced 48 hr after acute DFP. This may have resulted from a greater degree of receptor down-regulation consequent to such treatment with DFP.

CONCLUSION

It appears that central cholinergic neurotransmission is of importance in the regulation of SIA in rats. Though brain AChE is important in modulating the function of central cholinergic mechanism, postsynaptic cholinoceptor changes may also be involved in drug-induced modulation of this phenomenon.

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