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Anti-Aggressive Effect of a New Phenylpiperazine Compound (DU27716) on Hypothalamically Induced Behavioural Activities

A. M. VAN DER POEL, B. OLIVIER,* J. MOS, M. R. KRUK, W. MEELIS AND J. H. M. VAN AKEN*

*University Medical Centre, Department of Pharmacology, Sylvius Laboratorie Wassenaarseweg 72, 2333 AL Leiden, The Netherlands and *Department of Pharmacology, Duphar B.V., P.O. Box 2, 1380 AA Weesp, The Netherlands*

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VAN DER POEL, A. M., B. OLIVIER, J. MOS, M. R. KRUK, W. MEELIS AND J. H. M. VAN AKEN. *Anti-aggressive effect of a new phenylpiperazine compound (DU27716) on hypothalamically induced behavioural activities.* PHARMAC. BIOCHEM. BEHAV. **17**(1) 147–153, 1982.—Using the same hypothalamic electrodes, the following behaviour was evoked in male rats by electrical stimulation at roughly equal current intensities: attacks on a partner, teeth-chattering, switch-off behaviour and locomotion. Current thresholds were determined for each behaviour following the intraperitoneal administration of saline or DU27716, a new phenylpiperazine compound with interesting inhibitory effects on territorial and intermale aggression. DU27716 raised current thresholds for attack and teeth-chattering beginning at the lowest dose (4 mg/kg), whereas there was no effect on switch-off behaviour, and only a slight but significant effect on locomotion thresholds at the highest dose (8 mg/kg). The results provide support for the hypothesis that DU27716 possesses behaviourally selective, anti-aggressive properties, and illustrate the usefulness of hypothalamically induced behaviours as a pharmacological model.

Anti-aggressive compound Phenylpiperazine ESB Hypothalamus Male rats Aggression Locomotion Switch-off behaviour

THE testing of anti-aggressive properties of drugs on violent behaviour induced by immediate and direct alteration of central nervous activity in an environment where such behaviour is normally absent, may have some advantages. First, it allows the comparison of the potency of anti-aggressive drugs in terms of one simple physical parameter: the current intensity required to induce violent behaviour, rather than frequencies, latencies, intensities and/or patterning of the observed behavioural elements. Second, the model may provide clues which help to elucidate central nervous mechanisms underlying the anti-aggressive effects of drugs. Last, the model has some resemblance to behavioural disorders in which episodes of violent behaviour in otherwise peaceful subjects are attributed to sudden changes in CNS activity [1]. Aggression induced by electrical stimulation of the brain (ESB) may serve as such a model.

In rats electrical stimulation of a circumscribed area of the hypothalamus may induce intraspecific aggressive behaviour. The offensive nature of this behaviour is revealed by its close resemblance to the behaviour shown by a territory owner towards an intruder: threat, attack-jump, bite-attack, pilo-erection, teeth-chattering and short "aggressive" ultrasounds (50–60 kHz) [16]. In particular, the active appetitive behaviour preceding attack—without any provocative aggressive behaviour on the part of the opponent—strongly suggests that hypothalamic aggression is not defensive by nature. Moreover, ESB-induced and territorial aggression produce identical effects in the opponent: submissive behaviour and flight, long "submissive" ultrasounds (20–30 kHz) [2,10], and a characteristic pattern of wounds on head, neck and back of the opponent. In addition to aggression, hypothalamic stimulation may induce a variety of other behaviour as well, e.g., locomotor activity and teeth-chattering in non-social situations. If lever-pressing is made contingent on the interruption of stimulation, switch-off behaviour may develop. Studies on the relation between strength and duration of the electrical pulses used to induce the various behavioural activities [15] revealed that intraspecific aggression and locomotion, and intraspecific aggression and switch-off behaviour, are evoked by activa-

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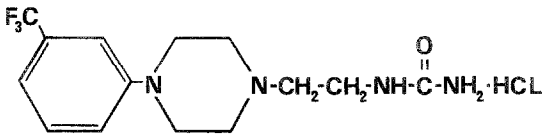
tion of different populations of neural elements in the hypothalamus [8]. No difference could be found when the strength-duration curves of intraspecific aggression and teeth-chattering were compared (unpublished observations). This diversity of induced behavioural effects therefore offers a unique possibility to test the specificity of drugs. For example, it is to be expected that a drug with specific anti-aggressive properties would impede stimulation-induced aggressive behaviour, like teeth-chattering and attack, while leaving other behaviour evoked at the same sites (e.g., locomotion and switch-off behaviour) relatively unaffected.

DU27716, a new phenylpiperazine derivative (Fig. 1) is claimed to be a specific anti-aggressive drug. This compound is to a great extent comparable chemically, pharmacologically and behaviourally to DU27725 [13]. The animal pharmacology (results to be published) showed high activity in several aggression tests: isolation-induced aggression in mice, ED_{50}=1.2 mg/kg, PO; group aggression in mice, ED_{50}=1.0 mg/kg, PO; territorial and intermale aggression in rats and mice, effective at 1–8 mg/kg IP. This anti-aggressive action was not caused by behaviourally aspecific effects like sedation, muscle relaxation or motor impairment. Ethological work on intermale aggression [12, 13, 14] further substantiated the similarity in action of the two drugs. DU27716, like DU27725, induced a specific decrease of offensive behaviour, while leaving the introductory social behaviour and other aspects, e.g., locomotor activity, unaffected. The results of the present experiment further support the specific anti-aggressive action of DU27716.

DU 27716



[2-[4-[3-(TRIFLUOROMETHYL) PHENYL]-1-PIPERAZINYL]ETHYL] UREA HYDROCHLORIDE

FIG. 1. Chemical structure and name of DU27716.

METHOD

More detailed descriptions of equipment and procedures can be found in previous reports from this group [6,7].

Experimental animals

Twenty five brown-eyed, beige-coloured male (CPB-WE-zob) rats were used as experimental animals. They were derived from the Central Breeding Institute for Laboratory Animals (CPB-TNO) at Zeist, The Netherlands. The animals were kept on a reversed day-night schedule (14 l/10 d), night starting at 8.00 a.m. They were operated upon at the age of 4 to 6 months, their weight then being between 350 and 500 g. Prior to operation the animals were housed in groups of 6 to 8 in large macrolon cages in quiet rooms at 22°C and 75% relative humidity. During this period they were accustomed to handling. Following the operation the rats were housed individually; food and water were always available. Male albino rats (CPB-WU: Wistar random) weighing between 180 and 200 grams served as sparring partners.

Surgery

Under Hypnorm-anaesthesia (0.1 ml per 100 g body weight) two bipolar stimulation electrodes were implanted bilaterally at the −2.5 mm D.V., 1.5 mm M.L., and 5.5 mm A.P. coordinates of the atlas of König and Klippel [4].

Stimulation Techniques

Trains of biphasic square-wave pulses with a phase duration of 0.2 msec and a phase interval of 12.5 msec were delivered by two Grass PSIU6 isolated constant-current sources driven by a Grass S88 stimulator. Voltage across the electrode and current intensity—i.e., the voltage across a 1 kΩ resistor in series with the electrode—was monitored. Automatic programming equipment allowed the changing of current intensity between stimulation trials. Great care was taken to equalize opposite phases in order to prevent net charge transfer and polarization of the electrode.

Initial Testing

Before behavioural testing commenced, animals were allowed a post-operative recovery period of one week. During the first testing of an electrode placement, animals were stimulated in the presence of a partner rat. Current was on for 120 sec and off for 60 sec periodically. Initial current intensity was set at 50 μA. In subsequent stimulation periods the current intensity was increased by 50 μA steps until either an upper limit of 400 μA was reached, or wild motor effects precluded further testing, or an attack on a partner was induced. Both left and right electrodes were tested. If possible, threshold current intensities for attack behaviour, teeth-chattering and locomotion were determined following the first testing.

Threshold Determination

Threshold current intensities for attack, teeth-chattering, locomotion and switch-off were determined according to a modification of up-and-down method of Dixon and Mood [3] proposed by Wetherill [19]. To find a threshold the current is switched on for 10 sec and switched off for 50 sec periodically. Starting from an arbitrary level the current intensity is decreased or increased each 10 sec trial by a fixed amount, depending on whether or not the behaviour of the animal met the criterion (respectively, 1 attack, 1 bout of teeth-chattering, 6 locomotion counts or 1 lever press per 10 sec trial, see below). Thus, the up-and-down method consists of increasing the current intensity by fixed steps until a particular behavioural response is induced, then decreasing it until the response is lost, increasing the current until the response to stimulation reappears, etc. A response change is defined as the mean current intensity of 2 succeeding trials to which the animal reacted differently. The threshold of a behaviour, i.e., the current intensity inducing that behaviour in 50% of the stimulation trials, was calculated from six subsequent response changes (Fig. 2). For each behaviour examined only one threshold per day was determined.

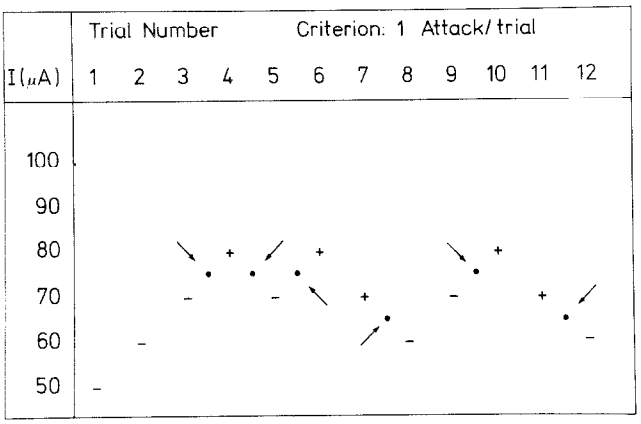


FIG. 2. Example of the procedure used to determine threshold current intensities. Vertical: current intensities; horizontal: successive 10 sec trials. Attack during trial: +; no attack during trial: −; arrows pointing to the dots indicate the response changes. The threshold in this particular example is 71.7 μA.

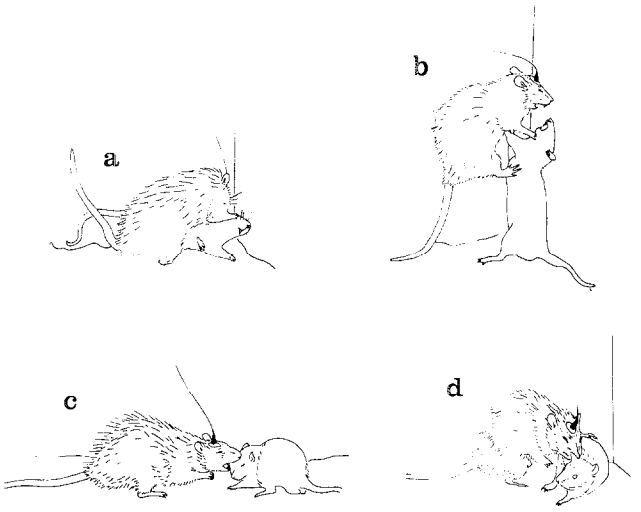


FIG. 3. Attacks induced by electrical stimulation in the hypothalamus of the rat. a: start of clinch fight; b: attack jump; c: bite attack; d: skin-pulling.

Behavioural Testing

Aggression. Aggression was tested in a Plexiglas cylinder (dia. 35 cm, height 45 cm) containing an inexperienced partner rat. Illumination was provided by 2 red 60 W light bulbs.

A trial was scored positively for aggression if the stimulated animal performed one or more of the following responses (see Fig. 3): clinch fight, attack jump with or without kicking with the hindlegs, strong or weak bite attack or skin-pulling [7]. Teeth-chattering and ultrasonic vocalizations were routinely monitored. Prior to the drug tests 6 thresholds for aggression were determined at approximately weekly intervals. Following each aggression test, the partners were killed by an overdose of ether, and the number, extent and localization of the wounds they had sustained were recorded.

Teeth-chattering. All electrodes producing aggression, also produced teeth-chattering upon stimulation. Chattering occurred in bouts lasting from part of a second to several seconds. It sometimes outlasted stimulation for several minutes, but mostly faded away within 30 sec. Post-stimulation teeth-chattering bouts sometimes alternated with teeth-grinding. Chattering was frequently accompanied by pilo-erection.

Thresholds for teeth-chattering were determined in the same cage as used for aggression tests, but in the absence of a partner. Before drug testing commenced, 5 thresholds for teeth-chattering were collected.

Locomotion. All but one electrode producing aggression and teeth-chattering, also produced locomotion upon stimulation. Locomotion was tested in a large cage, with a 50×100 cm floor area covered with an absorbent, felt-like material, 60 cm high walls on three sides and a 60×100 cm glass front window. The floor of this cage was divided into 8 squares of 25×25 cm each, by means of black lines on the substrate. During 10 sec-stimulation trials the number of squares traversed by the rat was counted. A count was obtained if the four paws of the rat completely passed the dividing or crossing lines between squares. A trial was scored positively for locomotion if 6 or more counts were obtained.

Switch-off behaviour. For switch-off behaviour animals were placed in a Plexiglas cylinder of the same dimensions as used for the testing of aggression and teeth-chattering. A lever protruded into the cage through a slit in the wall located 4 cm above the floor. Minimal training was required in order to measure switch-off behaviour. Training started the day after the second aggression threshold was determined. It consisted of 3 successive periods of 5 min in which the animals were allowed to switch off continuous stimulation for periods of 5 sec by pressing the lever. Additional presses during these 5 sec off-periods had no effect. Initial current intensity was set at the aggression threshold intensity last determined. The number of presses per 5 min was recorded. In 4 animals it was necessary to raise the current intensity in order to obtain the required minimal level of response (25–30 presses per 5 min). Learning was surprisingly fast at these current intensities. To illustrate this: the 8 remaining animals, trained at the unchanged initial current level, pressed at a mean rate of 21.6, 27.6 and 28.3 times per 5 min during the 3 consecutive periods of the first test, the last value being close to the mean rate obtained during later tests (32.7 presses per 5 min). Prior to the drug tests 4 additional switch-off tests were performed. In these tests lever-pressing rate was determined using the above procedure for only one period of 5 min. Immediately afterwards, the 10 sec on-50 sec off up-and-down design was used to determine a current threshold for switch-off behaviour. During the 10 sec stimulation trials pressing the lever interrupted the current for 1 sec. A trial was scored positively for switch-off behaviour if at least 1 lever press occurred.

Experimental Design

Initial testing yielded 22 "aggressive" electrodes in 13 animals. Following the initial threshold determinations 12 electrodes in 12 animals were selected on the basis of stability of their aggression thresholds. It so happened that there were 6 left and 6 right electrodes. The animals were subjected to 3 different treatments, according to a balanced design: they received either saline, or 4 or 8 mg/kg DU27716 IP.

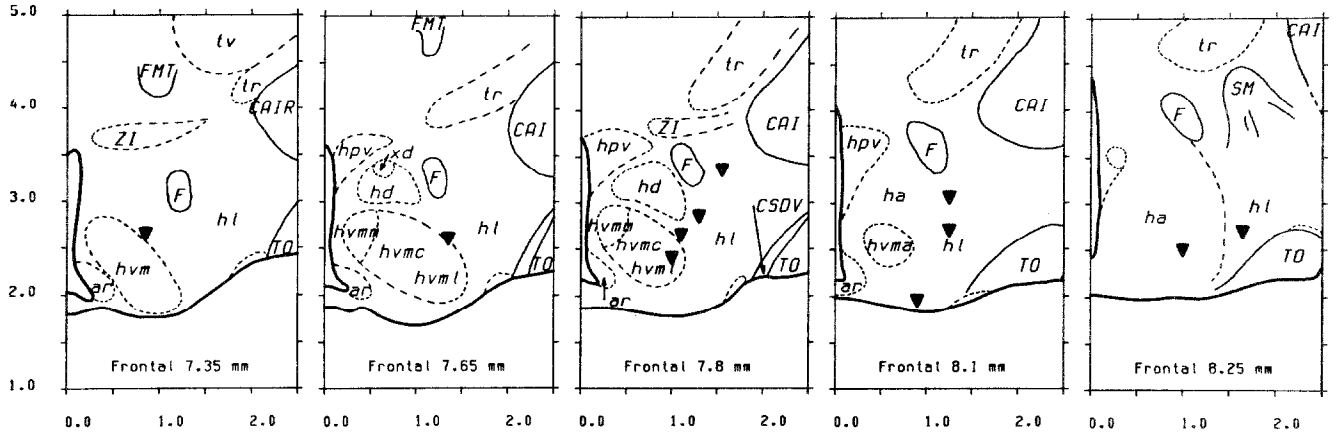


FIG. 4. Localization of the electrode tips plotted on frontal sections of our own atlas of the hypothalamus of the male CPB-WE-zob rat. Abbreviations: see König and Klippel [4].

Each dose was given twice, separated by at least one day of rest: the first administration of each dose was followed after 30 min by a threshold determination of aggression, which in turn was followed by tests of switch-off behaviour. The second administration of each dose was followed after 30 min by a test of locomotion, followed by a threshold determination of teeth-chattering. This procedure ensured that all tests were performed during the period of maximal activity of the drug. The mean threshold current levels of the last three pre-drug determinations were used as initial current levels in the drug tests. Depending on the mean current level, step sizes of 4 or 10 μA were used.

Drug Used

DU27716 dissolved in saline was used. Fresh solutions were prepared daily. All injections were given intraperitoneally in a volume of 0.1 ml/100 g body weight. Successive injection days were separated by at least 1 day of rest.

Statistical Evaluation

Treatment and order of treatment effects were analysed by multiple linear regression. In the absence of order of treatment effects, treatment effects were further evaluated by two-way analysis of variance. Contrasts between separate treatments were examined by Scheffé's method for multiple comparisons [17] which estimates 95% confidence limits (S-intervals). When a S-interval does not include zero, the difference is significant at least at the 0.05-level.

Histology

After completion of the experiments, the rats were anaesthetized and perfused with saline followed by 4% formaldehyde. After at least 14 days of storage in formaldehyde the brains were removed from the skulls. Freeze sections were stained according to the Fink-Heimer method. The localization of one electrode was lost in the process. The remaining electrodes were localized within the area described by Kruk [8], covering parts of the anterior, ventromedial and perifornical hypothalamus (see Fig. 4) where intraspecific aggression is readily obtainable by electrical stimulation.

RESULTS

For each electrode, the mean thresholds of the 3 tests preceding the drug tests were calculated for all behaviour examined. Overall means and ranges are shown in Table 1. The overall mean number of presses at aggression threshold intensity was 30.9 (range: 19.3–41.0).

In view of the large range of mean values obtained at different electrodes, the results of the drug tests are expressed as a percentage of these means. In addition, normalization of this kind facilitates comparison of the effects of the drug in the different test situations employed. The results are illustrated in Fig. 5.

Since the experiment was so designed that all animals received each dose level twice, it was deemed necessary first to evaluate possible trends resulting from repetition of treatments. This was accomplished by multiple linear regression analysis. No such trends (linear or non-linear) were found for any of the behavioural activities examined.

Analysis of variance revealed that treatment effects on aggression thresholds were significant (see Fig. 5a), $F(2,11)=11.32$, $p<0.01$. Contrasts between pairs of treatments were significant between saline and 8 mg/kg DU27716 (mean=57.7, S-interval=25.6–89.9), and between the 4 and 8 mg/kg-dosages (mean=36.1, S-interval=3.9–68.2). Since zero is not included in these S-intervals, the differences are significant at the 0.05-level.

The morphology of the attacks did not change with the administration of the drug (see Table 2). Even though appreciably more current was needed to induce attacks under the influence of the drug, no significant differences were detected in the number of the various forms of attack which were observed at threshold current intensities, $\chi^2(10)=9.55$, $p=0.48$. It is known [9] that in untreated animals stimulation at such high current intensities leads to increases in the frequency of attacks and a shift to more intense forms of attack, i.e., attack jumps and clinch fights.

There was also no apparent change in the distribution of the wounds over the various parts of the body of the partners (see Table 3). The absence of any clear-cut dose-dependent trends in the wound patterns reinforces the conclusion that there is no change in the defensive tactics of the partners and/or in the aiming of the attacks by the experimental animals under the influence of the drug.

TABLE 1

PRE-TREATMENT MEAN CURRENT THRESHOLD (μA) AND ITS RANGE (μA) OF FOUR HYPOTHALAMICALLY INDUCED BEHAVIOURAL ACTIVITIES

Behaviour	Number of Electrodes	Mean	Range
Aggression	12	54.9	32.2–145.6
Teeth-chattering	12	52.9	22.2–175.0
Switch-off	12	63.3	23.1–165.6
Locomotion	11	77.9	39.1–110.0

TABLE 2

NUMBER OF THE VARIOUS FORMS OF ATTACK, OBSERVED IN ALL TRIALS WITH ATTACK INDUCED BY LIMINAL STIMULATION OF THE HYPOTHALAMUS

Form of Attack	Saline	4 mg/kg	8 mg/kg
Clinch fight	6	3	7
Attack jump + kick	23	19	17
Attack jump − kick	16	16	17
Strong bite attack	43	44	47
Weak bite attack	23	18	8
Skin-pulling	1	2	1
Number of trials	60	56	60

TABLE 3

NUMBER OF BITES ON VARIOUS PARTS OF THE BODY OF PARTNER RATS IN ALL TRIALS WITH ATTACK AT LIMINAL CURRENT INTENSITY

Part of Body	Saline	4 mg/kg	8 mg/kg
Snout	0	2	2
Head	21	19	23
Forepaws	1	2	1
Frontal back	14	11	7
Caudal back	14	3	10
Belly	7	1	10
Hindpaws	0	0	2
Number of trials	60	56	60

Significant treatment effects on thresholds for teeth-chattering were observed (see Fig. 5b), $F(2,11)=9.35$, $p<0.002$. The effects of both the low and high dose were significantly different from saline control (mean=32.1, S-interval=4.1–60.1, and mean=44.8, S-interval=16.8–72.8 respectively). The difference between low and high dose was not significant. Analysis of variance yielded no significant results either for switch-off thresholds (see Fig. 5d), $F(2,11)=3.41$, $p>0.05$, or for the number of presses at a fixed current intensity (see Fig. 5e), $F(2,11)=1.06$, $p>0.35$.

As regards locomotion there were again significant treatment effects (see Fig. 5c), $F(2,10)=8.02$, $p<0.005$. However, only the comparison of the highest dose with saline control yielded a significant contrast (mean=17.0, S-interval=5.7–28.3).

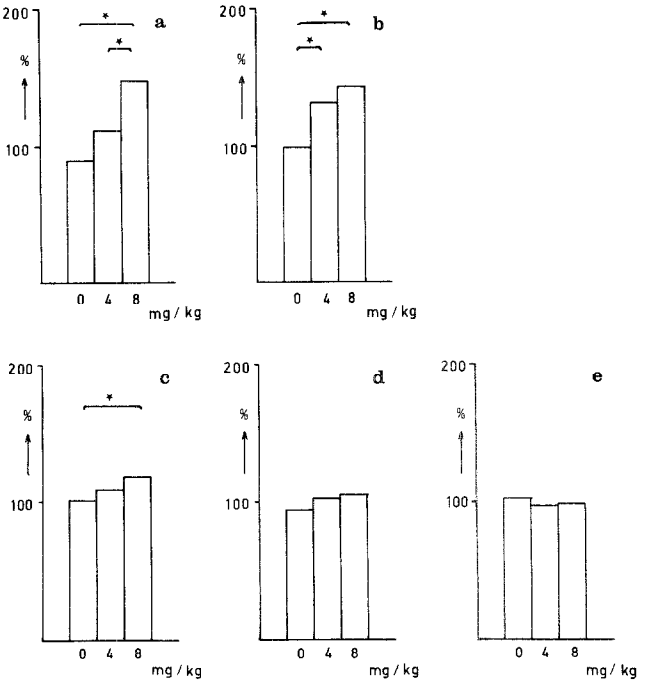


FIG. 5. Effects of DU27716 on threshold current intensities of hypothalamically induced behaviours. Results are expressed as percentages of previously determined base levels. a: attack; b: teeth-chattering; c: locomotion; d: switch-off thresholds; e: switch-off rate of lever-pressing. *$p<0.05$.

If the effect of the drug is expressed as the difference between the percentage scores of saline control and the highest dose (Fig. 5), the effect on attack thresholds is significantly larger than the effects on locomotion (contrast=44.1, S-interval=6.7–81.4), and switch-off thresholds (contrast=51.4, S-interval=14.0–88.7), but does not differ from the effects on teeth-chattering (contrast=12.7, S-interval=−24.6–50.1). The effect on teeth-chattering is significantly different from the effect on switch-off thresholds (contrast=38.6, S-interval=1.3–76.0), but not from the effect on locomotion (contrast=31.3, S-interval=−6.0–68.7). The contrast between the treatment effects on locomotion and switch-off thresholds is not significant.

DISCUSSION

The absence of order of treatment effects indicates that tolerance or sensitization to the behavioural effects of DU27716 did not develop under the treatment regime of the present study. The most prominent effect of the drug is the concurrent inhibition of hypothalamically induced teeth-chattering, and attacks on a partner, although there are minor differences in the degree of inhibition: at the low dose of 4 mg/kg the effect on teeth-chattering is already quite pronounced (see Fig. 5), whereas compared to teeth-chattering the effect on attacking seems stronger at the high dose of 8 mg/kg. At 8 mg/kg it can even be contrasted with the effects on locomotion. In line with the view that teeth-chattering and attack belong to one behavioural system—a view based on ethological is well as neurophysiological grounds (see introductory paragraphs)—both effects point in

the same direction: dose-dependent inhibition of hypothalamic aggression.

Following DU27716 the nature of the attacks induced by electrical stimulation of the hypothalamus does not change. This is revealed by the absence of effects on the morphology of the attacks as well as on the total number and distribution of the wounds inflicted on the partners. The different catagories of attack discerned (see Table 2) represent different intensities of an aggressive tendency; clinch fight being the most intense form and skin-pulling being the least intense form. This view is based on differences in effort exerted by the animals in the various forms of attack, on differences in damaging power (clinch fights often result in rather large, open wounds particularly on back and flanks, whereas incisors seldom penetrate the skin in skin-pulling), and on differences in the amount of current needed to induce the various forms of attack in one electrode (the more intense forms needing more current). Since the intensity of the attacks is not changed after the administration of the drug, although more current is needed to induce them, it follows that DU27716 exerts a graded inhibitory influence on hypothalamically induced aggression, as opposed to an all-or-nothing kind of inhibition, which should have led to threshold increases combined with attacks of increased intensity.

Van der Poel and Remmelts [18] demonstrated that behavioural effects of drugs in a social situation may not be limited to the treated animal: the behaviour of untreated partners may also be altered. As suggested by Olivier [13] it is therefore often difficult to decide whether behavioural effects of drugs measured in a social situation are primary effects, or secondary effects brought about by changed behaviour of the partner. Since a threshold procedure was used throughout in the present study, and thus the output of behaviour by the experimental animals under the various treatment conditions was kept deliberately constant (see also Tables 2 and 3), there is no need to take partner-induced secondary effects into account.

In the dosages employed in the present study, DU27716 has at best only minimal effects on switch-off behaviour: this applies to the rate as well as to the threshold for lever-pressing. This is an important result, since it rules out any interpretation of the effect on aggression as being due to pain, frustration or other aversive consequences of hypothalamic stimulation. It also rules out the possibility that the behavioural effects of this drug are mediated by an eventual general depressant effect.

DU27716 has a slight, inhibitory effect on ESB-induced locomotion, reaching significance only at the highest dose. This result is in conflict with results obtained in a earlier study (Olivier, unpublished results). In that study locomotion was measured in an open field. No effects were noted at comparable dose levels. This might mean that the two tests measure different things, e.g., locomotor activity per se or exploration.

The results of the present study provide the first hint that hypothalamically induced aggression might serve as an appropriate pharmacological model to test anti-aggressive properties of drugs. First, with this drug DU27716 the model appears as selective as other models currently in use for this purpose (e.g., territorial and intermale aggression). Furthermore, although the dose-response relationships have not yet been fully explored, the sensitivity appears to be roughly equal (ED_{50} somewhere between 1 to 8 mg/kg IP for all models tested so far). Since the results can be expressed as a single parameter, the current intensity needed to evoke the behavioural activities, data-gathering and evaluation are much simpler than the more complicated ethological tests of territorial and intermale aggression [13].

Kruk and Van der Poel [8] and Mos [11] have elaborated the view that both switch-off and locomotion are behavioural output from one or more hypothalamic networks, different from the hypothalamic network which produces teeth-chattering and attack upon activation by electrical stimulation. The differential pharmacological effects on switch-off, locomotion and aggression obtained in this study indicate that this model may indeed allow a pharmacological differentiation and characterization of hypothalamically activated neural systems with different behavioural outputs, and hence may be of help in the elucidation of the mechanism of action of anti-aggressive drugs.

It also justifies the conclusion that DU27716 suppresses aggression induced by electrical stimulation of the hypothalamus in a behaviourally specific way. This result extends and further substantiates the conclusions of earlier studies demonstrating the selective anti-aggressive properties of the new phenylpiperazine family of drugs to which DU27716 belongs [12, 13, 14].

REFERENCES

1. Bandler, R., R. Halliday and S. Abeyewardene. Centrally-elicited aggressive behavior in the cat: a changing view. In: *Limbic Epilepsy and the Dyscontrol Syndrome*, edited by M. Girgis and L. G. Kiloh. Amsterdam: Elsevier/North-Holland Biomedical Press, 1980, pp. 93–106.
2. Corrigan, J. G. and K. J. Flanelly. Ultrasonic vocalizations of defeated male rats. *J. comp. physiol. Psychol.* **93:** 105–115, 1979.
3. Dixon, W. J. and A. M. Mood. A method for obtaining and analyzing sensitivity data. *J. Am. statist. Ass.* **43:** 109–126, 1948.
4. König, J. F. R. and R. A. Klippel. *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem.* Baltimore: Williams and Wilkins, 1963.
5. Kruk, M. R. Origins of hypothalamic aggression in the rat. Thesis, Leiden, 1981.
6. Kruk, M. R., P. Kuiper and W. Meelis. An air pressure-operated commutator system for electrical brain stimulation in a fighting rat. *Physiol. Behav.* **21:** 125–127, 1978.
7. Kruk, M. R., A. M. Van der Poel and T. P. De Vos-Frerichs. The induction of aggressive behaviour by electrical stimulation in the hypothalamus of male rats. *Behaviour* **70:** 292–321, 1979.
8. Kruk, M. R. and A. M. Van der Poel. Is there evidence for a neural correlate of an aggressive behavioural system in the hypothalamus of the rat? In: *Adaptive Capabilities of the Nervous System. Progress in Brain Research,* vol. 53, edited by P. S. McConnell, G. J. Boer, H. J. Romijn, N. E. Van de Poll and M. A. Corner. Amsterdam: Elsevier/North-Holland Biomedical Press, 1980, pp. 385–390.
9. Kruk, M. R., W. Meelis, A. M. Van der Poel and J. Mos. Electrical stimulation as a tool to trace physiological properties of the hypothalamic network in aggression. In: *The Biology of Aggression.* NATO Advanced Study Institute Series, edited by P. F. Brain and D. Benton. Alphen aan den Rijn, The Netherlands, and Rockville, MD: Sijthoff and Noordhoff, 1981, pp. 383–395.
10. Lore, R., K. Flanelly and P. Farina. Ultrasounds produced by rats accompany decreases in intraspecific fighting. *Aggress. Behav.* **2:** 175–181, 1976.

11. Mos, J. The midbrain central gray and aggression in male rats. Thesis, Leiden, 1981.
12. Olivier, B. A new anti-aggressive compound: ethological studies. *Aggress. Behav.* **6:** 262–263, 1980.
13. Olivier, B. Selective anti-aggressive properties of DU27725: Ethological analyses of intermale and territorial aggression in the male rat. *Pharmac. Biochem. Behav.* **14:** Suppl. 1, 61–77, 1981.
14. Olivier, B. and F. C. Hillen. Behavioural and pharmacological effects of a new anti-aggressive compound. *Prog. Neuropsychopharmac.* Vol. 4, Suppl. 1, 270–271, 1980.
15. Ranck, J. B. Which elements are excited in electrical stimulation of mammalian central nervous system: a review. *Brain Res.* **98:** 417–440, 1975.
16. Sales, G. D. Ultrasound and aggressive behaviour in rats and other small mammals. *Anim. Behav.* **20:** 88–100, 1972.
17. Scheffé, H. *The Analysis of Variance.* London: John Wiley, 1964.
18. Van der Poel, A. M. and M. Remmelts. The effect of anticholinergics on the behaviour of the rat in a solitary and in a social situation. *Archs int. Pharmacodyn. Thér.* **189:** 394–396, 1971.
19. Wetherill, G. B. Sequential estimation of points on quantal response curves. In: *Sequential Methods in Statistics,* edited by G. Barrie and G. B. Wetherill. London: Methuen and Co., 1966, pp. 162–179.