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Anticonflict Effects of Low Doses of the Dopamine Agonist Apomorphine in the Rat

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HJORTH, S , J A ENGEL AND A CARLSSON *Anticonflict effects of low doses of the dopamine agonist apomorphine in the rat* PHARMACOL BIOCHEM BEHAV **24**(2) 237–240, 1986 —The effects of low, "autoreceptor doses (3 13–100 μg/kg, SC) of the dopamine (DA) agonist apomorphine were investigated in a modified Vogel's conflict paradigm The compound was found to exert a marked, dose-dependent increase in the number of shocks taken in the conflict situation (maximum ~230% of control responding, obtained at 12 5 μg/kg), thus indicating an anxiolytic action However, the dose-response curve was biphasic, inversely U-shaped, with the highest dose tried actually suppressing the punished response rate to below control levels Neither low- nor high-dose apomorphine modified the rats' drinking "motivation" (glucose intake after 48 hr of water deprivation) On the other hand, while unaltered by 12 5 μg/kg, the pain threshold tended to be lowered by 100 μg/kg It is suggested that the anxiolytic-like action of apomorphine might be due to central DA autoreceptor stimulation, possibly in limbic/cortical forebrain regions The conflict-promoting effect seen at 100 μg/kg is likely related to the concomitantly elicited hyperalgesia The possibility of developing novel DA-modulating agents for the treatment of anxiety is raised

Low-dose apomorphine Anticonflict Rat Central dopamine autoreceptor Antianxiety agents

THE vast majority of research concerning central neurotransmitter systems and anxiety states has been directed towards γ-aminobutyric acid (GABA), serotonin (5-HT) and noradrenaline (NA) neurons, their interplay and the effects of anxiolytic drugs on those systems as well as on the specific benzodiazepine binding sites (see, e g , [23,24]) On the other hand, the possible role of dopamine (DA) in anxiety is largely overlooked However, small doses of DA antagonists have been reported to exert anxiolytic effects in certain clinical conditions [6,22], even though neuroleptics in general are considered to be minimally efficacious in the treatment of anxiety states Interestingly, as evident from the literature, small doses of the DA agonist apomorphine can also act beneficially in a variety of CNS disorders associated with excitation, agitation and/or anxiety (e g , [9,17]) It is well established that this agent can either suppress or promote the activity of central DA synapses depending on the dose and the experimental conditions applied [3] In an attempt to provide some further insight into the possible relation between central DA transmission and anxiety, we therefore considered it worthwhile to scrutinize the effects of apomorphine in a commonly used animal test model of anxiety—the Vogel conflict paradigm [27]

METHOD

Male rats of the Sprague-Dawley strain (ALAB, Sollentuna, Sweden), weighing 180–250 g, were used in the experiments The animals were kept under controlled standard environmental conditions (temperature 25°C, humidity 60%, lights on 5 30 a m –5 30 p m) for at least one week after arrival in the department until used in the experiments Laboratory chow (R3, Astra-Ewos, Sodertalje, Sweden) and tap water were allowed ad lib prior to commencement of the experimental procedure

Conflict testing was carried out as previously described [5] On the first day of the experiment the rats were adapted for 10 minutes to the test chamber This was a Plexiglas box (inner dimensions 30×24×20 cm) enclosed in a soundproof cage, and equipped with a grid floor of stainless steel bars and a drinking bottle containing a 5 5% (w/v) glucose solution An electric shock (0 16 mA, delivered for 2 sec every 3rd sec by means of a commercially available shock generator Grason Stadler E1064) could be applied between the spout of the drinking bottle and the grid floor The shock intensity (0 16 mA) was chosen on the basis of previous experiments, establishing a level of responding (drinking attempt) in control animals that would allow the detection of potential anti- as well as "pro"-conflict drug actions, i e , increases and decreases, respectively, in the number of shocks taken during the session

After the initial adaptation the animals were water-deprived for 24 hr and then placed in the test chamber for a further 5 min adaptation, during which they had free access to the drinking bottle Thereafter they were allowed a 30-min free drinking session in their home cage After another 24 hr water-deprivation period, the rats were again placed in the test chamber and were allowed to drink the glucose solution for 30 sec Immediately thereafter every subsequent drinking attempt was punished with an electric shock The number of shocks accepted during a 10-min experimental session was recorded To minimize diurnal variations in behavior all experiments were carried out between noon and 3 00 p m The animals were used only once in these studies

Pain sensitivity was assessed in the conflict testing chamber, but with the electrical shock delivered through the

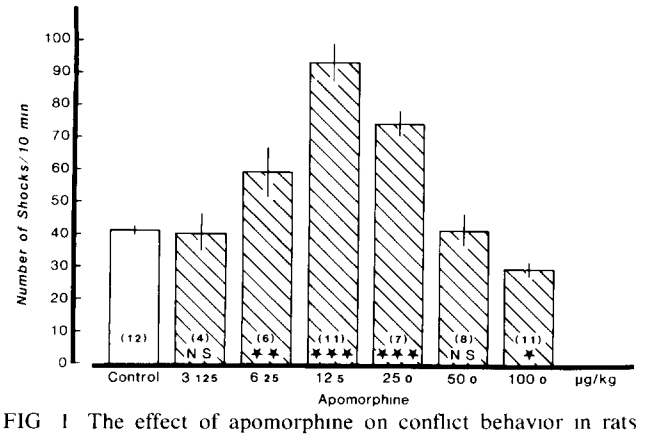

FIG 1 The effect of apomorphine on conflict behavior in rats Apomorphine (3 13–100 µg/kg SC) or vehicle was given to the rats 10 min prior to placement in the conflict test chamber The total number of shocks accepted during the 10-min test session was recorded The means±S E M of 4–12 observations are shown One-way ANOVA, $F(6,52)=27\ 75$, $p<0\ 001$, followed by t-test was used for statistical comparisons with the control (vehicle) group *$p<0\ 05$, **$p<0\ 01$, ***$p<0\ 001$, N S =not significant, $p>0\ 05$

grid floor instead of the drinking spout (Grason Stadler E1064 shock generator) The conflict testing chamber and the shock generator-regulating device were situated in separate rooms The shock intensity was manually raised stepwise by one assistant in the "shock-generator room," from 0 05 mA (steps 0 05, 0 06, 0 08, 0 10, 0 13, 0 16, 0 20, 0 25, 0 30, 0 40 mA, shock duration 2 sec, inter-shock interval 10 sec) until a reaction indicative of perceived pain (jerk/jump or similar) was observed by a second assistant in the "conflict-testing room " The pain reaction occurred typically between 0 13 and 0 16 mA in vehicle-treated rats The observer was blind to the treatment of the animals and to the current intensity applied

The drinking "motivation" was also estimated in the group of animals previously used in the pain sensitivity test For these measurements the rats were deprived of water for 48 hr and subsequently placed into individual "home" cages with free access to 5 5% glucose solution The amount of liquid drunk during a 2 hr-period was then determined

A treadmill (Rotarod) with a diameter of 6 cm and a rotational speed of 8 rpm was used to assess the possibility that apomorphine negatively affected muscular tone or coordination The animals were trained daily to walk on the treadmill in 10-minute sessions A total of 3–4 training sessions were typically required until they were able to pursue the treadmill walking for 300 consecutive seconds without falling off Animals that failed to fulfill this criterion in two subsequent training sessions were not used in the experiments (usually 1–2 out of ten rats) The test session was performed 24–36 hr after the last training session Apomorphine or vehicle was injected SC in the neck 10 min prior to the test The amount of time (sec) spent walking on the treadmill before falling off, during the 300-sec test session was recorded

Apomorphine HCl×$^1/_2$ H_2O (Sandoz A G , Basel, Switzerland) was dissolved in 0 9% NaCl with a few grains of ascorbic acid (~0 1% of final volume) to prevent oxidation Apomorphine or the vehicle was administered SC in the neck, in a volume of 2 ml/kg

Statistical treatment of the experimental data was carried out by means of one-way analysis of variance followed by t-test Probabilities of less than 5% were considered significant

TABLE 1

EFFECT OF APOMORPHINE ON PAIN SENSITIVITY

Apomorphine (µg/kg, SC)	Threshold current (mA)	n
0 (Vehicle)	0 15 ± 0 006	6
12 5	0 14 ± 0 013	7
100	0 12 ± 0 008	7

Rats were given apomorphine (12 5 or 100 µg/kg, SC) or vehicle Ten min later they were individually placed into the conflict test chambers Electrical shock of step-wise increasing intensity was then delivered through the grid floor until an indication (jerk/jump or similar) of perceived pain was observed (for further details, see the Method section) Shown is the mean (±S E M) threshold current (mA) required to obtain an observable reaction in the animals Statistics One-way ANOVA $F(2,17)=2\ 478$, no significant difference between treatment groups ($p>0\ 05$)

RESULTS

As shown in Fig 1, apomorphine exerted a dose-dependent anticonflict effect in the modified Vogel conflict paradigm The maximal anticonflict effect was found at 12 5 µg/kg (230% of control responding) and the minimal effective dose ($p<0\ 01$) was 6 25 µg/kg However, the dose-response curve was biphasic, inversely U-shaped, with a decreased number of accepted shocks after higher doses of apomorphine (>25 µg/kg) The highest dose tested, 100 µg/kg, reduced the number of shocks/10 min significantly below control levels ($p<0\ 05$)

In separate experiments it was shown that the threshold for reacting to electric shock was unaltered by 12 5, while somewhat, though non-significantly ($p<0\ 1$), decreased by 100 µg/kg (SC) of apomorphine (Table 1) The 2 hr-intake of 5 5% glucose solution after 48 hr of water deprivation was not modified ($F(2,17)=0\ 418$, N S) by either dose of the drug (vehicle controls 22 5±1 0 ml (mean±S E M , n=6), apomorphine 12 5 µg/kg, SC 21 9±0 8 ml (n=7), apomorphine 100 µg/kg, SC 23 3±1 5 ml (n=7)) Likewise, as assessed by rotarod performance, the motor coordination was unaffected by these doses of apomorphine (controls 300±0 sec, n=5, apomorphine 12 5 µg/kg, SC 300±0 sec, n=6, apomorphine 100 µg/kg, SC 300±0 sec, n=6)

DISCUSSION

The present study shows that low doses of apomorphine increased the number of shocks taken in the conflict model, thus indicating an anxiolytic action The effective doses closely agree with a previous preliminary report (Hyslop *et al* [25]) However, the dose-response curve was inversely U-shaped, with the highest dose tried actually reducing the number of accepted shocks to below control levels, possibly related to an increase in pain sensitivity *(vide infra)*

When administered in low doses, the DA agonist apomorphine preferentially stimulates central DA autoreceptors—thereby reducing the activity (cell firing, release, synthesis and turnover) in dopaminergic neurons (see [23]) Behavioral consequences considered related to an im-

pairment of, in particular the meso-limbic/cortical, DA transmission are locomotor hypoactivity and suppression of exploration in experimental animals [10,15] When given at high doses, apomorphine instead elicits locomotor hyperactivity and various stereotyped behaviors These behavioral effects are thought associated with increased postsynaptic DA-receptor stimulation in limbic and striatal parts of brain, respectively (e g , [13,14]) Interestingly, a striking correspondence is evident when comparing the doses of apomorphine inducing hypo (but not hyper-) motility (see, e g , [18]) with those producing anti-conflict effects From the present data it is clearly not possible to identify the exact neuroanatomical locus where apomorphine produces its anxiolytic-like action It is, however, tempting to speculate that this action—like the locomotor hypoactivity—is mediated via an inhibition of DA neuronal activity, tentatively in meso-cortical/limbic pathways (*vide infra*)

The possibility that the anxiolytic action of the benzodiazepines may in part be mediated by the dopaminergic system has been discussed by, e g , Taylor *et al* [25] Thus the benzodiazepines have been shown to reduce dopamine turnover Furthermore, the increase in, particularly meso-cortical, DA turnover seen after, e g , electrical foot-shock [19,26] can be antagonized by classical anxiolytics like diazepam (reversed by a benzodiazepine antagonist, [19]) and chlordiazepoxide, also at doses which *per se* lack effect on DA turnover [7, 16, 21] In the interpretation of these findings a benzodiazepine-induced promotion of GABAergic influences upon ventral tegmental (A10) DA neurons (projecting to, e g , frontal cortex and nucleus accumbens) was specifically implicated (for further discussion see, e g , [25]) The anticonflict action of apomorphine may thus be considered within this context DA neurons projecting to prefrontal cortical areas and activated by footshock ([19] cf above) are claimed to lack synthesis- and nerve impulse-modulating DA autoreceptors (e g , [2] but see [1,8]), but may possess terminal release-modulating DA autoreceptors [1, 12, 30] Thus, if the anxiolytic-like effect of apomorphine reflects an action at the level of these neurons it might possibly involve an autoreceptor-mediated reduction of the DA release Needless to say, these tentative explanations remain to be verified

Although still in the "autoreceptor range, the two highest doses of apomorphine, 50 and 100 μg/kg (SC), did not release the drinking suppression maintained by electrical shock In fact, the latter dose reduced the number of shocks accepted below control level in the conflict situation However, the present data demonstrate that, while unaltered by 12 5 μg/kg (SC), the pain sensitivity tends to be somewhat enhanced by the 100 μg/kg (SC) apomorphine dose This is in agreement with Paalzow and Paalzow [20], who showed a dose-dependent hyperalgesia (25–100 μg/kg, SC) with ED_{50} and maximum effects at 36 and 100 μg/kg, respectively, for apomorphine Higher doses (>400 μg/kg, SC) were analgesic These investigators proposed DA autoreceptor stimulation as a tentative explanation for the observed lowering of pain threshold In comparing the apomorphine actions a possible rationalization of the marked difference in maximum effective doses may be that the activity levels are different in dopaminergic systems involved in the control of conflict behavior and pain reactivity Regional variations in apparent DA autoreceptor sensitivity have also been claimed from both *in-vivo* and *in-vitro* studies (e g , [28,29]) In summary, the apparent "pro-conflict ' effect of apomorphine might be related to a lowered pain threshold On the other hand, this hyperalgesic effect of apomorphine is unlikely to be responsible for its anti-conflict action in the present behavioral paradigm, and may indeed even result in an underestimation of the latter effect

It might be argued that apomorphine-induced alterations in drinking "motivation" or motor co-ordination are responsible for its actions in the conflict situation However, the 2 hr-intake of 5 5% glucose solution after water deprivation for 48 hr was not affected by the compound, thereby precluding the first alternative (cf also [4]) Similarly, neither gross behavioural observations nor treadmill experiments disclosed any sign of impaired motor function following either dose of apomorphine (12 5 or 100 μg/kg, SC) As noted above, none of the apomorphine doses tested elicited any hyperactivity or stereotypies (gross behavioral observations) Even though predominantly autoreceptor active, the highest dose (100 μg/kg, SC) approaches the range where clearcut stimulation of postsynaptic DA receptors occurs (cf , e g , [18]) Thus, it cannot be entirely excluded that other behaviors competing with the drinking (resulting in a decreased number of drinking attempts and, thus, shocks taken) and/or an elevation of serum corticosterone levels [11] may, in addition to the hyperalgesic effect, contribute to the conflict-promoting action of this dose of apomorphine Alternatively, a more direct relation between potential postsynaptic DA receptor stimulation and the "pro"-conflict effect of apomorphine could also be conceived

As mentioned in the Introduction, there is evidence that treatments aimed at attenuating central dopaminergic neurotransmission can be beneficial in the management of certain states of anxiety, especially when seen in connection with psychotic conditions such as schizophrenia Interestingly, in a recent double-blind study on schizophrenics, Ferrier *et al* [9] noted a significant and specific antianxiety effect of low-dose apomorphine in the acute, but not in the chronic psychotic condition Reviewing the literature, Taylor *et al* [25] suggested that dopamine is implicated in the etiology and expression of anxiety The present findings would appear consistent with this proposal Furthermore, given the well-known side-effect propensity of classical benzodiazepines and neuroleptics (e g , ataxia and extrapyramidal disturbances, respectively) the possibility of developing new DA-modulating compounds with higher anxioselectivity may be raised

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