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Blockade of Apomorphine's Discriminative Stimulus Properties: Relation to Neuroleptic Activity in Neuropharmacological and Biochemical Assays[1,2]

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COLPAERT, F. C., J. E. M. F. LEYSEN, C. J. E. NIEMEGEERS AND P. A. J. JANSSEN. *Blockade of apomorphine's discriminative stimulus properties: relation to neuroleptic activity in neuropharmacological and biochemical assays.* PHARMAC. BIOCHEM. BEHAV. 5(6) 671–679, 1976. – Using a food-reinforced two-lever operant conditioning procedure, rats were trained to discriminate 0.16 mg/kg apomorphine from saline. Eight neuroleptics of the phenothiazine, butyrophenone or diphenylbutylamine type were investigated for their ability to antagonize the discriminative stimulus properties of apomorphine. The same drugs were also assayed for in vivo antagonism of apomorphine-induced stereotyped behavior as well as for in vitro inhibition of stereospecific ^{3}H-haloperidol binding in rat striatal tissue preparations. The data are consistent with the hypothesis that apomorphine exerts its discriminative stimulus properties by a mechanism similar to that underlying its stereotypogenic action. The loci involved in these two phenomena are likely to be distinct.

Apomorphine Phenothiazine Butyrophenone Diphenylbutylamine Discriminative stimulus Stereotyped behavior Binding sites

IT has been shown [7,8] that apomorphine is able to produce a discriminative stimulus complex (DSC) in rats. Evidence for this was obtained by a procedure specifically designed [6] to assess the properties which provide the opportunity for drugs to serve a discriminative stimulus function in a food-reinforced two-response operant conditioning paradigm.

In view of the present pharmacological and biochemical knowledge on apomorphine (for review, see: [9]) it seemed reasonable to hypothesize [8] that the action underlying the apomorphine-DSC might consist of the dopamine-mimicking activity of the drug at specific dopamine binding sites. In support of this hypothesis it was found that blockade of these binding sites [1] by the highly specific neuroleptic, haloperidol [13], effectively antagonizes the perception of apomorphine injection.

The studies presented here sought to explore two problems concerning the nature of the apomorphine-DSC. One is whether blockers of dopamine binding sites other than haloperidol would similarly antagonize this DSC. This issue relates directly to the mode of action involved in the discriminative stimulus properties of apomorphine.

The second problem resides in the locus of this action. The fact that haloperidol antagonizes the apomorphine-DSC does not indicate the DSC to originate centrally, as haloperidol may antagonize various central but also peripheral effects of apomorphine [9]. To this end, the potency of a chemically heterogenous series of neuroleptics to antagonize the apomorphine-DSC was determined, and comparative studies were performed to investigate the activity of the same drugs in the brain. This activity was measured, in vivo, by assessing the antagonism of apomorphine-induced stereotyped behavior which is characteristic for neuroleptic drugs [15, 16, 20]; in vitro measurement was obtained in biochemical experiments assessing the affinity of these neuroleptic drugs for specific binding sites [10,26] in rat striatal tissue preparations.

EXPERIMENT 1

This experiment aimed to determine the antagonist effects of drugs able to block dopamine binding sites, on the apomorphine-DSC in rats. Eight neuroleptics of the phenothiazine, butyrophenone or diphenylbutylamine type

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were studied for this purpose. The selection was based upon pharmacological [16, 17, 18, 19, 20, 24] and biochemical [1] data on the relative specificity of these drugs as regards their blocking action at dopamine binding sites.

METHOD

The procedures applied in the drug discrimination experiments have been described in full elsewhere [4,6].

Animals

The experimental animals were 11 male Wistar rats weighing 210 ± 10 g (age: 7 to 8 weeks) at the beginning of the experiments. They were housed in individual living cages, stored in a continuously illuminated and air conditioned room (21 ± 1°C; R. H. 65 ± 5 %). Tap water was available freely; access to dry powdered standard laboratory food was limited to 2 hr a day, as specified below.

Apparatus

Standard small animal test cages (Model E 10–10; Coulbourn Instr., Inc.) were used as experimental chambers; they were programmed by solid state logic modules and fitted with a house light and two levers. Between the two levers, a food pellet receptacle was mounted 2 cm above the cage floor.

Training Procedure

Discrimination training started after habituation to the experimental conditions and initial shaping. The discriminative stimuli consisted of two pairs of subcutaneous injections; one was saline plus saline (SS), the other saline plus the training drug (SD), i. e. 0.16 mg/kg apomorphine. The first of these injections was always given 60 min before the daily session; the second was given 30 min after the first one.

Depending on whether the animal received either the SS- or the SD-treatment, it could obtain reinforcement by pressing either the saline lever (SL) or the drug lever (DL) respectively. That is, after every tenth press (fixed-ratio 10) on the correct lever a pellet (45 mg P. J. Noyes Precision Food Pellets) was delivered through a pellet dispenser. Responses on the incorrect lever (i.e. the DL after SS- and the SL after SD-treatment) produced no programmed consequences. The lever assignments were SL: right, DL: left in 6 animals, and SL: left, DL: right in the other 5 rats.

The number of responses made on either lever before the first food pellet was obtained (and, thus, before 10 responses were made on the correct lever) was recorded (symbol: FRF); the FRF value reflects the accuracy of the animals' lever selection. Fifteen min after the rat had been put into the test cage, the session was stopped and all correct and incorrect responses made in the course of the 15-min period were recorded (symbol: TR).

After the session, the animal was transferred to its living cage; one hr later it was allowed to feed freely for 2 hr. The daily sessions were held between 8 and 11 a.m. On week-end days (when no training was given), free access to food was allowed between 10 and 12 a.m.

Every week, each rat was run once a day on 5 consecutive days. Daily SS- or SD-treatments were given according to two weekly alternating sequences. The first sequence was: SD–SS–SS–SD–SD; the second sequence was: SS–SD–SD–SS–SS. The learning criterion was reached when the animals' FRF did not exceed 12 for at least 15 consecutive training sessions. When this was accomplished, the rat was submitted to the stimulus generalization experiments described below. It should be noted that possible position preferences in rats so trained, do not affect the discrimination because the learning criterion requires the animal to perform correctly at both levers.

Stimulus Generalization Experiments

During the period following training, test sessions were held on Wednesdays and Fridays; on the 3 remaining days of the week, the above described SS- and SD-training conditions were continued in order to provide appropriate base-line data on the reliability of lever selection and on total response output.

For test sessions, the animal was injected, 60 min before session, with one of the neuroleptics listed below; 30 min later it was given a standard (0.16 mg/kg) apomorphine injection. Following this treatment, the rat was placed in the test cage, and the lever on which it first totaled 10 responses was determined. This lever will be further referred to as the selected lever. Once this choice had been established, the rat obtained a first food pellet, and subsequent reinforcement was made contingent upon pressing (fixed-ratio 10) the selected lever. Pressing the alternative lever produced no programmed consequences.

Each rat received the 3 doses of all 8 neuroleptics in a randomized sequence. Test sessions were interspaced by at least one SS- or SD-control session; the TR on such control sessions was evaluated for possible carry over effects of test drugs. In the event of such effects, no test session was held on the subsequent test day, and testing was continued only after control responding had reached base-line level again. Maintenance conditions were similar to those applied during the training period.

Drugs and Doses

The neuroleptic drugs studied here were azaperone, benperidol, droperidol, haloperidol, pimozide and spiperone (expressed as the base), chlorpromazine HCl and trifluperidol HCl. The latter 2 drugs were dissolved in water, all other drugs were dissolved in 1 eq. H_2T.

All drugs were injected subcutaneously at a constant volume of 1 ml/100 g body weight. The doses studied were selected on the basis of preliminary experiments in similarly trained rats.

RESULTS

Base-line Data

The 11 animals participating in the present experiments required a median number of 41 training sessions (not including the 10 criterion sessions) in order to meet the learning criterion.

After the training period, all rats were run 3 times a week on further (SS- or SD-) standard sessions, and the baseline data thus obtained were similar to those described earlier [8] for rats trained as described here. All animals reliably selected the appropriate lever after both distinct standard treatments, i.e. the SL following SS-, and the DL following SD-treatment. Incorrect lever selections occurred only rarely, and each individual rat reached a highly significant level (one-tailed $p < 0.001$; binomial test [28])

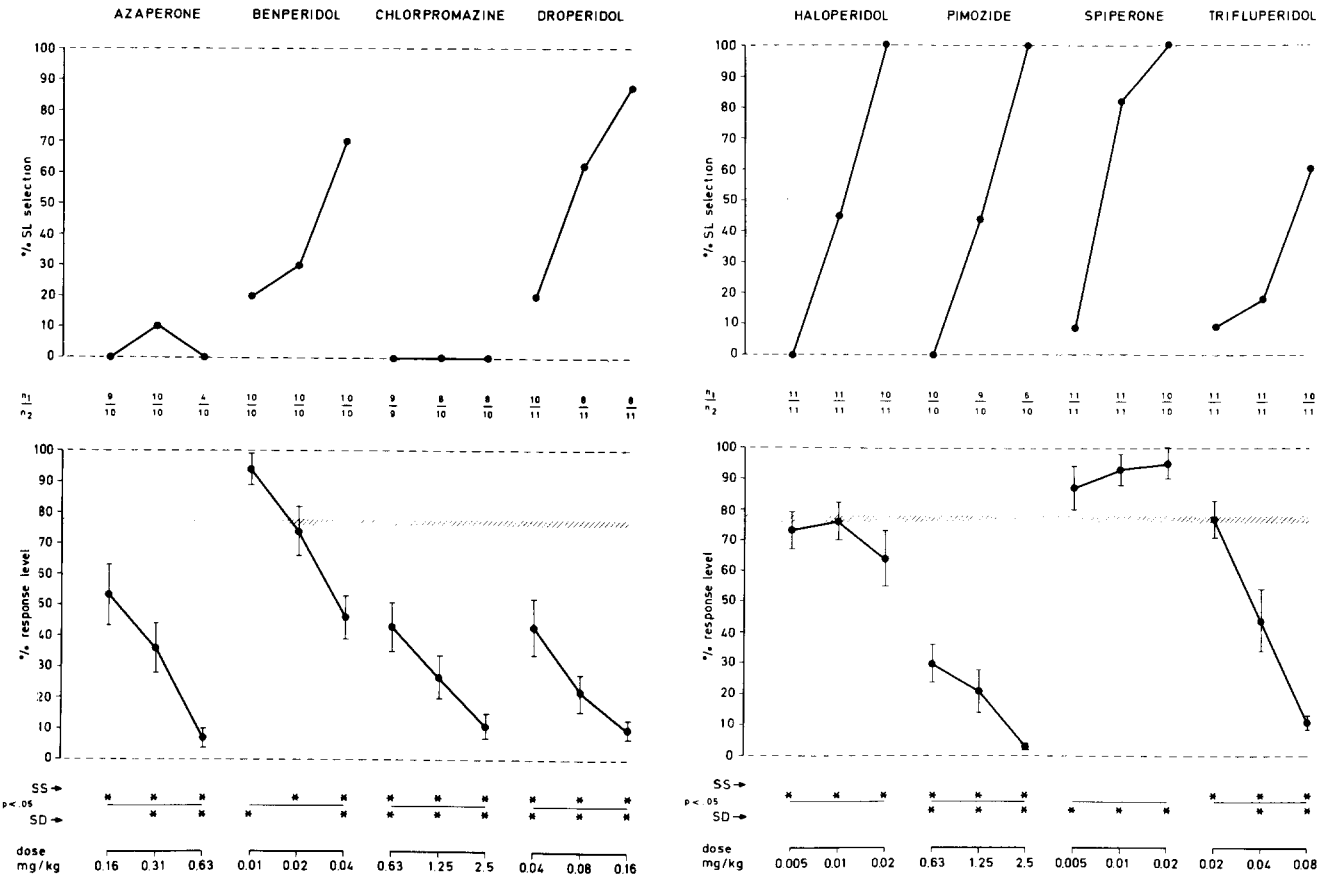

FIG. 1. (A) Effects of azaperone, benperidol, chlorpromazine and droperidol on the perception of the apomorphine-DSC. Percent SL selection represents the percentage of rats selecting the saline lever. n_1/n_2 pertains to the number of rats which made sufficient responses to demonstrate lever selection, out of the total number of rats tested. Percent response level is expressed as the mean (± 1 SEM) and refers to the total number of responses emitted after test drug injection, expressed as a percentage of responding after SS-treatment. Response level 100% corresponds to SS-standard sessions; the dashed area indicates the response level in SD-standard sessions. The asterisks above the line at the bottom pertain to significance of difference (Wilcoxon test; [28]) between test- and SS-standard sessions; those below the line pertain to differences between test- and SD-standard sessions. (B) Effects of haloperidol, pimozide, spiperone and trifluperidol on the perception of the apomorphine-DSC. Symbols as in Fig. 1(A).

of correct lever selection. The median (and 95% confidence limits: C. L.) FRF value was 10 (10 – 10) for each animal individually under either standard treatment. The accuracy of further responding, as documented by the percentage of TR on the correct lever, was virtually perfect [median: 100 (100 – 100)]. The individual mean (± 1 SEM) TR output ranged from 1013 (± 27.1) to 1530 (± 20.6) after SS- and from 620 (± 27.1) to 1305 (± 24.4) after SD-treatment. The individual mean response level (i.e. TR after SD, expressed as a percentage of the TR on the most recently preceding SS-treatment) varied from 53% (± 2.2) to 99% (± 4.1), and the response decrease produced by 0.16 mg/kg apomorphine was statistically significant (one-tailed $p<0.001$; Wilcoxon-test; [28]).

Stimulus Generalization Experiments

The results of the stimulus generalization experiments are summarized in Fig. 1. 2 animals died in the course of the experiments, and the data hence pertain to the 9 to 11 rats tested per dose of each drug. The data on FRF values and on the percentage of TR on the selected lever are not represented, because no case of significant deterioration of these values occurred. Test data on TR were compared (two-tailed $p\leq 0.05$; Wilcoxon test [28]) to the means of TR values obtained on SS- and SD-control sessions during the week of test as well as during the one preceding and the one following that week. The latter procedure accounts for slight intraindividual shifts in TR control performance, and enhances reproducibility of test results.

After pretreatment with either azaperone (0.16 to 0.63 mg/kg) or chlorpromazine (0.63 to 2.5 mg/kg) before apomorphine (0.16 mg/kg), all rats always selected the DL; the one rat that selected the SL upon 0.31 mg/kg azaperone failed to respond upon 0.63 mg/kg of the same drug. Both compounds produced a dose-related decrease of responding, and the response level following 0.31 to 0.63 mg/kg azaperone and 0.63 to 2.5 mg/kg chlorpromazine was significantly below SD-level. Benperidol (0.01 to 0.04 mg/kg) pretreatment caused the animals to select the SL in a dose-dependent way, thus indicating that the perception of the DSC otherwise evident after the standard apo-

morphine dose, had been antagonized. At the dose of 0.01 mg/kg, benperidol also significantly counteracted the response decrease due to apomorphine, and responding did not differ from the SS-level. The latter effect disappeared at the 0.02 mg/kg dose of the drug; following 0.04 mg/kg, responding was significantly below the SD-level.

Droperidol (0.04 to 0.16 mg/kg) and pimozide (0.63 to 2.5 mg/kg) appeared to act very similarly; both compounds produced a dose-related SL selection, and caused significant response decreasing effects. Haloperidol (0.005 to 0.02 mg/kg) antagonized DL-selection, and all animals selected the SL upon 0.02 mg/kg of the drug. However, haloperidol appeared to be the only drug which, at the dose level used, failed to exert any significant effect on response output.

Spiperone (0.005 to 0.02 mg/kg) was found to be most potent antagonist of the apomorphine-DSC. The drug counteracted the response decrease otherwise produced by the standard 0.16 mg/kg apomorphine dose; most remarkably, the response level, if anything, appeared to increase rather than to decrease with increasing doses of spiperone.

Finally, trifluperidol also caused dose-related SL selection, and significantly decreased responding at the doses 0.04 and 0.08 mg/kg.

The slopes of the dose-response curves for apomorphine-DSC antagonism (SL selection) by benperidol, droperidol, haloperidol, pimozide, spiperone, and trifluperidol were quite similar; the test for parallelism of slopes [21] indicated that no slope differed significantly (two-tailed $p < 0.05$) from any other slope, the slope functions varying between 1.32 (haloperidol) and 2.07 (benperidol). In contrast, all 6 ED_{50} values (Table 1) for apomorphine-DSC antagonism differed significantly ($p<0.05$) from one another; the only exception was the non-significant difference between the ED_{50} values of trifluperidol (0.062 mg/kg) and droperidol (0.069 mg/kg).

DISCUSSION

The present data on haloperidol (ED_{50}: 0.010 mg/kg: Table 1) are in close agreement with earlier results [8] obtained in similarly trained rats (ED_{50}: 0.008 mg/kg), thus indicating that the procedures used here provide highly reliable results [6].

There is convincing pharmacological [16, 17, 18, 19, 20, 24] and biochemical [1] evidence that benperidol, droperidol, haloperidol, pimozide, spiperone and trifluperidol are able to block dopamine binding sites, although there exist marked differences between these drugs as regards their specificity in this respect [1,16]. The finding that pretreatment with these drugs prevents the perception of the apomorphine-DSC corroborates the hypothesis [8] that the discriminative stimulus properties of apomorphine might depend critically upon its agonist effects at dopamine binding sites. The failure of azaperone and chlorpromazine, at doses which nearly completely suppressed responding, to antagonize the apomorphine-DSC is consistent with the fact that these compounds exert sedative and adrenolytic effects at doses lower than or equal to those required to block dopamine binding sites [1,16].

Two aspects of the present data deserve particular

TABLE 1

EFFECTS OF 8 NEUROLEPTIC DRUGS ON APOMORPHINE-INDUCED DISCRIMINATIVE STIMULUS COMPLEX, STEREOTYPED BEHAVIOR AND EMESIS, AS WELL AS ON STEREOSPECIFIC ^{3}H-HALOPERIDOL BINDING

Source	Experiment 1		Experiment 2	Experiment 3	Literature [17, 19, 24]
Species	Rat (in vivo)		Rat (in vivo)	Rat striatal tissue (in vitro)	Dog (in vivo)
Phenomenon studied	Apomorphine-DSC antagonism		Apomorphine-SB antagonism	Specific ^{3}H-haloperidol binding	Apomorphine-emesis antagonism
Compound	ED_{50} (mg/kg)	Response level (%)	ED_{50} (mg/kg)	IC_{50} (M)	ED_{50} (mg/kg)
Spiperone	0.0076 (0.0059-0.0098)	90	0.021 (0.015-0.031)	3.46×10^{-9} (± 1.07)	0.00049 (0.00032-0.00076)
Haloperidol	0.010 (0.0081-0.013)	75	0.017 (0.011-0.026)	6.70×10^{-9} (± 1.15)	0.015 (0.012-0.019)
Benperidol	0.025 (0.016-0.040)	65	0.010 (0.0059-0.019)	1.86×10^{-9} (± 0.13)	0.00059 (0.00032-0.0011)
Trifluperidol	0.062 (0.040-0.095)	23	0.019 (0.012-0.028)	4.42×10^{-9} (± 0.87)	0.0088 (0.0031-0.025)
Droperidol	0.069 (0.044-0.11)	27	0.014 (0.0087-0.022)	5.76×10^{-9} (± 1.61)	0.0012 (0.00052-0.0028)
Pimozide	1.30 (1.00-1.69)	14	0.12 (0.068-0.21)	7.86×10^{-9} (± 2.14)	0.025 (0.017-0.039)
Azaperone	>0.63	0	0.77 (0.36-1.66)	3.88×10^{-7} (± 2.10)	1.25 (0.730-2.15)
Chlorpromazine	>2.50	0	0.26 (0.16-0.43)	2.17×10^{-7} (± 0.35)	0.54 (0.32-0.91)

The in vivo data are expressed as ED_{50} values and 95% confidence limits [21]; the in vitro data are represented as the mean (± 1 SEM) of 3 x 3 determinations per drug concentration.

attention. One is the relative potency with which the neuroleptics studied here antagonize the apomorphine-DSC. This potency does not seem to reflect any thus far known pharmacological effect of these drugs and hence constitutes essentially new information on neuroleptic activity. The second point relates to the relative specificity with which these compounds exert this antagonist activity. Thus, some of them (i.e. droperidol, pimozide, trifluperidol) produced a dose-related response decrease at the dose range required to antagonize the apomorphine-DSC. Haloperidol exerted no significant effect, whereas benperidol (0.01 mg/kg) and spiperone (0.005 to 0.02 mg/kg) restored responding otherwise decreased by 0.16 mg/kg apomorphine. These response rate increasing and decreasing effects have formerly [8] been explained as being due to two independent factors, i.e. antagonism of apomorphine-produced response decrease and intrinsic rate decreasing effects of neuroleptics. Although this explanation remains valid, it does not account for the relative specificity of drugs as regards the relation between response rate altering effects on the one hand and apomorphine-DSC antagonism on the other. Thus, for example, pimozide blocks central dopamine binding sites at doses far below those required to evidence sedative and adrenolytic effects [1,19]. Were the apomorphine-DSC based upon the agonist effects of the drug at central dopamine binding sites, then pimozide would be expected to antagonize this DSC at relatively low doses producing very little or no decreasing effects on response rate. Hence, pimozide would be predicted to be a very specific antagonist of the apomorphine-DSC. This expectation is not corroborated by the present results; in fact, pimozide actually appears to be the least specific of all 6 drugs that were found to antagonize the apomorphine-DSC (Fig. 2).

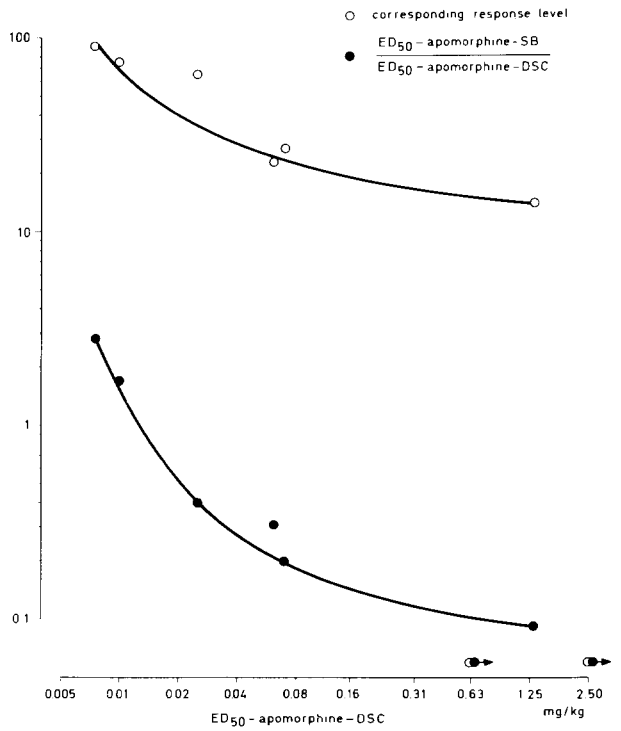

FIG. 2. Double-logarithmic plot of the relation between apomorphine-DSC antagonism and corresponding response level on the one hand, and the ratio of apomorphine-SB antagonism to apomorphine-DSC antagonism on the other.

EXPERIMENT 2

The stereotyped behavior (SB) induced by apomorphine is generally considered [9] to be due to the drug's ability to mimick endogenous dopamine at its central binding sites. Antagonism of apomorphine-SB [15], accordingly constitutes evidence for blockade of central dopamine binding sites, and the procedure is currently used in pharmacological research on neuroleptic drugs [16,20].

In view of the results of experiment 1, it seemed valuable to assess this blocking activity in a parallel *in vivo* experiment in such a way that the conditions as regards, for example, species, route, and time would contribute minimally to the variance of the data.

METHOD

The procedures employed here are similar to those commonly used in related studies [15, 16, 20].

Animals

The experimental animals were male Wistar rats weighing 240 ± 10 g (age: 8 to 9 weeks) at the time of experiments. The day before use, they were transferred from their rearing quarters to the air-conditioned laboratory (21 ± 1° C; R. H. 65 ± 5 %). The animals were used only once.

Procedure

After being deprived of food overnight, but having free access to tap water, the rats were injected subcutaneously with either saline or a drug solution, and were put singly into isolated perspex observation cages (19 × 12 × 15 cm). 30 min later, the animals received an injection of 1.25 mg/kg apomorphine into the tail vein, and were observed in the observation cages for a 1 hr period. During this period, apomorphine-SB (i.e. chewing, sniffing, or licking) was observed every 5 min and scores were assigned according to the following system.

Score 0: total absence of chewing, sniffing or licking;
Score 1: occasional occurrence of one of these activities;
Score 2: continuous presence of one of these activities;
Score 3: continuous and maximally intense chewing, sniffing, or licking.

The experiments were carried out by an unbiased trained technician using coded bottles containing either saline or drug solution. In each experiment, each rat was treated with a different compound or saline (control), and this was repeated on different days until 5 rats per dose had been tested. These precautions were taken to randomized day-to-day variability, to minimize systematic errors and to improve quantitative comparison of the activity of the compounds.

Drugs and Doses

Apomorphine HCl was injected intravenously at a volume of 0.2 ml/100 g body weight; all other drug solutions and saline were subcutaneously injected at a constant volume of 1 ml/100 g body weight.

The doses tested were derived from the geometrical series 0.01, 0.02, . . ., 320, 640 mg/kg, and ranged from 0.04 to 5 mg/kg for azaperone, from 0.0025 to 0.04 mg/kg for benperidol, from 0.04 to 1.25 mg/kg for chlor-

promazine, from 0.005 to 0.16 mg/kg for droperidol, from 0.01 to 0.08 mg/kg for haloperidol, from 0.02 to 0.63 mg/kg for pimozide, and from 0.005 to 0.08 mg/kg for spiperone and trifluperidol. These were the dose ranges which, on the basis of earlier studies [15, 16, 19, 20, 24], were expected to yield the data required to establish appropriate ED_{50} values.

The forms of drugs and their solutions were identical to those indicated for Experiment 1.

RESULTS AND DISCUSSION

The data obtained in saline pretreated (control) rats (n = 500) were similar to the previously reported [15, 16, 20] behavioral effects of an intravenous 1.25 mg/kg apomorphine challenge in rats. In short, 492 (98.4 %) control animals were assigned a score ≥ 2 on at least 7 subsequent occasions (corresponding to 35 min) in the course of the 1 hr observation period; 8 controls (1.6 %) were given this score on 6, and none on 5 or less occasions. On the basis of these control data, the following criterion was established: a drug pretreated rat was considered as significantly affected by the drug if it showed SB (Score ≥ 2) for a period equal to or less than 30 min. Computations of ED_{50} values were based on this criterion.

The ED_{50} values (Table 1) thus established are in good agreement with earlier reports [16, 19, 20, 24] on neuroleptic antagonism of apomorphine-SB in rats, and merit little comment at this point. The minor differences between the present and earlier determinations of these ED_{50} values are undoubtedly due to differences in route and time of drug pretreatment. The conditions applied in Experiment 2 were selected so as to match as closely as possible those applied in Experiment 1.

Statistical evaluation [21] showed that azaperone and chlorpromazine are significantly less potent than all other drugs, the only exception being the non-significant difference between pimozide and chlorpromazine. Spiperone was significantly more potent than benperidol, droperidol and pimozide, and the latter drug was significantly less potent than benperidol, droperidol, haloperidol and trifluperidol. Other differences did not reach significance ($p>0.05$). A more elaborate discussion of Experiment 2 is given in the General Discussion section.

EXPERIMENT 3

Experiment 3 aimed to provide a biochemical in vitro measurement of neuroleptic activity in rat brain tissue. As neuroleptic drugs are presumed [29] to exert their antipsychotic effect by virtue of their ability to block central dopamine binding sites, it was felt worthwhile to establish the relative affinity of the neuroleptics studied in the former experiments, for these specific binding sites. For this purpose, these drugs were investigated for their ability to inhibit stereospecific binding of labeled haloperidol to rat striatal tissue.

METHOD

Animals

Upon their arrival from the rearing quarters, female Wistar rats (body weight: 150 ± 10 g; age: 6 weeks) were killed by decapitation, and their brains rapidly removed. The corpora striata were then dissected as described elsewhere [12].

Tissue Preparation

Tissue was prepared according to procedures described elsewhere [10].

After rinsing in cold Tris (hydroxy-methyl)-aminomethan-HCl buffer (0.05 M; pH 7.7), the tissue was homogenized in 40 volumes of the buffer using an Ultra Turrax homogenizer. The homogenate was then centrifuged for 30 min at 35,000 G, the supernatant was discarded, and the pellet was washed twice with the original volume of cold buffer and then centrifuged for a further 20 min at 35,000 G. The final pellet was suspended in 80 volumes cold Tris-HCl buffer (0.05 M, pH 7.6) containing 10 μM pargyline, 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$. Thereafter, the tissue preparation was incubated for 5 min at 37°C and then cooled in ice water.

Binding Experiment

The incubation mixture was composed of (a) 2 ml of the tissue preparation corresponding to 25 mg original tissue, (b) 0.100 ml ^{3}H-haloperidol solution containing 0.043 μCi of specific activity of 10.5 Ci per mmole, and (c) 0.100 ml of a solution containing either 4.4 nmole (+)-butaclamol or 0.44 nmole (-)-butaclamol or drug. After incubation with simultaneous shaking for 10 min at 37°C, the mixture was rapidly filtered under suction through a Whatman FG/B glass fiber filter and rinsed twice with 5 ml cold Tris-HCl buffer. The filter was then transferred to a counting vial, 1 ml 1 % Triton X-100 and 10 ml Instagel were added; this was followed by vigorous shaking for 10 min. Finally, radioactivity was estimated in a Packard Tri-Carb liquid scintillation spectrometer.

Using the same tissue preparation, 5 concentrations of each drug were tested, and each incubation was run in triplicate. IC_{50} values (i.e., the drug concentration inhibiting the stereospecific ^{3}H-haloperidol binding by 50%) were estimated graphically on a double-logarithmical plot (abscissa: drug concentration; ordinate: radioactivity collected on the filter paper). 100% activity was defined as the amount of radioactivity obtained after incubation in the presence of (-)-butaclamol; 0 % activity as the radioactivity remaining on the filter after incubation in the presence of (+)-butaclamol. In this way, IC_{50} values were independently determined 3 times, using different tissue preparations and different drug solutions.

Drugs and Concentrations

All neuroleptic drugs (forms as in Experiments 1 and 2) were applied as aqueous solutions.

The concentrations studied ranged from 10^{-9} to 10^{-5} M for chlorpromazine and azaperone, and from 10^{-10} to 10^{-6} M for the other 6 drugs.

RESULTS AND DISCUSSION

The specificity of in vitro ^{3}H-haloperidol binding to striatal tissue preparations is characterized by stereospecificity, as is indicated by the difference between the inhibitory potencies of (+)-butaclamol and its pharmacologically inactive (−)-isomer [10,26]. The stereospecific binding thus defined is saturable, and can be inhibited by various dopamine agonists and antagonists [10,26].

In the present experiment, the use of a labeled ligand

(^{3}H-haloperidol) with very high specific activity allowed to work at an extremely low concentration (2×10^{-9} M). As a result, the stereospecific binding amounts to 60% of the radioactivity collected on the filter; this represents 12,000 DPM in control experiments. The IC_{50} values for stereospecific ^{3}H-haloperidol binding are given in Table 1. Benperidol (IC_{50}: 1.86×10^{-9} M) appeared to be the most potent inhibitor of the 8 drugs studied; its IC_{50} value differed [21] slightly though significantly ($p < 0.05$) from that of trifluperidol, droperidol, haloperidol and pimozide. No significant differences in potency were found between spiperone and the drugs of the latter group. The most marked differences (ratio $\approx$ 100) were those between chlorpromazine and azaperone on the one hand, and the 6 other drugs on the other; however, the two former drugs did not differ significantly from one another.

It follows from these data, that large quantitative differences exist between sedative and typically antipsychotic neuroleptics as regards their ability to bind to stereospecific binding sites in vitro. The relevance of this finding with respect to the in vivo pharmacological activity of these drugs will be dealt with in the General Discussion section.

GENERAL DISCUSSION

The data obtained in the pharmacological and biochemical investigations reported here are summarized in Table 1. For each of the 8 neuroleptic drugs studied, this table indicates: (a) the ED_{50} value for antagonism of the apomorphine-DSC; (b) the graphically estimated response level which corresponds to the latter ED_{50}: (c) the ED_{50} value for antagonism of apomorphine-SB; (d) the IC_{50} value for inhibition of stereospecific ^{3}H-haloperidol binding using rat striatal tissue preparations. In addition, earlier data (expressed in terms of ED_{50} values) on antagonism of apomorphine-induced emesis in dogs were also included. In the latter experiments. the neuroleptic drugs (or saline) were subcutaneously injected 1 hr before the subcutaneous apomorphine (0.31 mg/kg) challenge; the occurrence of emesis was then observed for up to 1 hr after this challenge [14, 17, 22]. The correlations (r_S) referred to below pertain to the Spearman Rank correlation coefficient [28]; azaperone is not included in some of these computations because its juxtaposition with reference to some of the other drugs studied is left undetermined by Experiment 1.

The finding (Experiment 1) that drugs which block the binding sites for endogenous dopamine [1] also antagonize the apomorphine-DSC corroborates the hypothesis [8] that this DSC is based upon apomorphine's ability [9], to mimic the agonist effects of dopamine at its specific binding sites. For the sake of parsimony it must be assumed, therefore, that the mechanism underlying apomorphine-DSC antagonism by neuroleptic drugs consists of blockade of dopamine binding sites.

The data contained in Table 1 further emphasize the intriguing relation between apomorphine-DSC antagonism and the corresponding response level; in fact, there exists a very close negative correlation ($r_S = -0.96$; $p<0.01$) between these two measurements. The only juxtaposition which is not identical in these two samples is the one between trifluperidol and droperidol. This does not seem to be of any relevance, because the ED_{50} values of these compounds do not differ significantly, and because the error introduced by graphical estimation of corresponding response levels may very well exceed the extent to which the rate decreasing effects of the two drugs actually differ.

Experiment 2 relates to antagonism of apomorphine-SB; the induction as well as the antagonism of this SB is generally thought [9] to be based upon specific central actions of apomorphine and neuroleptic drugs respectively. The rationale underlying Experiment 2 was as follows: were the apomorphine-DSC, like apomorphine-SB, of central origin, then the potency with which neuroleptic drugs antagonize both phenomena might very well be similar. The present data (Table 1) fail to corroborate this possibility; despite far-reaching methodological similarities, antagonism of the apomorphine-DSC is not significantly correlated ($r_S = 0.46$; $p>0.05$) with antagonism of apomorphine-SB. Most remarkably, however, there is a perfect correlation ($r_S = -1.0$; $p < 0.01$) between the ED_{50} value for apomorphine-DSC antagonism on the one hand, and the ratio of apomorphine-SB antagonism to apomorphine-DSC antagonism on the other (Fig. 2). Thus, the more potent a neuroleptic drug is with respect to apomorphine-DSC antagonism, the higher is this potency as compared with the drug's ability to antagonize apomorphine-SB. Although its nature remains obscure, the correlation itself may readily account for the above-mentioned correlation between apomorphine-DSC antagonism and corresponding response level. That is, at the dose levels required to antagonize apomorphine- or amphetamine-SB, the neuroleptics studied here also exert marked rate-decreasing effects on various types of conditioned behavior, such as avoidance responding in a shuttle box [16, 19, 20, 24] and lever pressing for electrical stimulation of the brain [32]. It can be inferred from this, that a drug (e.g. spiperone) which antagonizes the apomorphine-DSC at a dose considerably (ratio: 2.8) lower than its ED_{50} for apomorphine-DSC antagonism, will produce relatively little intrinsic effect on food-reinforced lever pressing. The opposite holds true for drugs for which this relation between doses is reversed (e.g. pimozide). Allowing for experimental error, the validity of this prediction on the basis of the curves depicted in Fig. 2 appears to be sufficiently well grounded to explain the relation between apomorphine-DSC antagonism and response rate decreasing effects in Experiment 1.

To further examine the possible relations between apomorphine-DSC antagonism and other in vivo measurements of apomorphine antagonism, the antiemetic potency of the drugs studied here was also included in Table 1. In contrast to the stereotypogenic action of the drug [9], apomorphine-induced emesis does not require the drug to actually penetrate the brain tissue; it depends [2, 25, 27, 30, 31] upon apomorphine's agonist effects at binding sites localized in the chemoreceptor trigger zone of the fourth ventricle (the area postrema: [3]). Among numerous other drugs, neuroleptics typically antagonize thus induced emesis [14, 17, 22], and the antiemetic potency of neuroleptic drugs in dogs possesses significant predictive value for antipsychotic potency in humans [23].

It follows, then, that neuroleptic antagonism of apomorphine-induced emesis shows some correlation with antagonism of the apomorphine-DSC ($r_S = 0.75$; $p<0.05$) and, also, with antagonism of apomorphine-SB ($r_S = 0.74$; $p < 0.05$). These correlations themselves are not necessarily highly informative; they may very well reflect mere drug potency and, hence, contribute little to the understanding of the phenomena studied here. With respect to apomorphine-DSC antagonism, however, it is interesting to

consider that this drug effect does not correlate with another one (i.e. apomorphine-SB antagonism) assessed in otherwise equivalent experimental conditions, whereas it does show significant correlation with an effect (i.e. apomorphine-emesis antagonism) assessed in quite different (e.g. species) conditions. This result underlines the significance of the lack of correlation between antagonism of apomorphine-DSC and -SB, and constitutes further reason to suggest that the sites of action involved in these two phenomena are distinct.

The results (Table 1) of Experiment 3 indicate that in vitro stereospecific ^{3}H-haloperidol binding shows some correlation with all 3 in vivo assays. The coefficients are: 0.71 ($p < 0.05$) for apomorphine-DSC antagonism, 0.79 ($p < 0.05$) for apomorphine-SB antagonism, and 0.95 ($p < 0.01$) for apomorphine-emesis antagonism. To give an interpretation of these findings seems unwarranted at this stage because of the large methodological gap between the in vivo and the in vitro experiments. A careful examination of these correlations requires the study of much larger series of structurally related compounds covering a wide range of pharmacological activity (Leysen, in preparation). The major point made by Experiment 3 however is, that chlorpromazine and azaperone, as compared with the 6 other drugs studied, possess much lower affinity for specific ^{3}H-haloperidol binding sites. The thus established large difference in potency, which clearly separates both drugs from the others, is again apparent in all 3 in vivo assays (Table 1). This consistency thus constitutes further support for the hypothesis that, in as far as biochemical mechanism is concerned, the activity of neuroleptic drugs in these assays is basically determined by their ability to occupy the binding sites at which apomorphine acts to produce its agonist effects (i.e. DSC, SB, and emesis). There seems to be general agreement that these sites are also the specific binding sites for endogenous dopamine [10,26].

As the data discussed here fail to provide any argument for there being a basic difference in mechanism by which apomorphine produces a DSC and exerts other agonist effects, it is reasonable to consider the possibility that the loci involved are distinct. This is the more attractive, as difference in locus is also the most likely explanation for the discrepancies between neuroleptic antagonism of apomorphine-SB and -emesis. The presumed binding sites involved in these two phenomena are localized within brain tissue (i.g. corpus striatum; [9]) and on the surface of the ventricular wall [3] respectively. Upon systemic injection and absorption into the blood, the penetration of drugs into brain tissue and cerebrospinal fluid is governed by a number of passive and active transport mechanisms which partially differ for both compartments [11]. The absence of any correlation between antagonism of apomorphine-DSC and -SB then suggests that the former effect, unlike the latter, might not require the drug to penetrate the brain tissue. It is interesting to note, at this point, that drug discrimination learning can be established on the basis of central [4] as well as of peripheral [5] effects of drugs.

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