Br. J. Pharmac. (1981), **73**, 843–852

COCAINE CUE IN PIGEONS: TIME COURSE STUDIES AND GENERALIZATION TO STRUCTURALLY RELATED COMPOUNDS (NORCOCAINE, WIN 35,428 AND 35,065-2) AND (+)-AMPHETAMINE

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1 Pigeons trained to discriminate between the presence or absence of effects induced by cocaine hydrochloride (5.6 mg/kg) were tested for generalization with norcocaine and two phenyltropane analogues (WIN 35,428 and WIN 35,065-2). Separate dose-effect curves were obtained at different intervals after the injections so that possible changes both in potency and duration of action could be evaluated.

2 Results showed that all of these drugs fully generalized to cocaine. The order of potency was WIN 35,428 > norcocaine > WIN 35,065-2 > cocaine when tested either at 15 or 60 min after injection. The cocaine-like effects were strongest for all drugs when tested 15 min after injection as compared to the tests at the 60 min interval. The decay of the cocaine-like stimulus effects occurred at about the same rate.

3 Apomorphine (0.3, 0.56 and 1 mg/kg), morphine (3 and 5.6 mg/kg), Δ^9-tetrahydrocannabinol (0.3 and 0.56 mg/kg), and lysergic acid diethylamide (LSD-25, 0.056 and 0.1 mg/kg) did not induce more than 30% cocaine appropriate responses. (+)-Amphetamine produced 73% and 85% cocaine appropriate responses depending on the injection-test interval used, 15 and 30 min respectively.

4 The amphetamine homologue, *para*-hydroxyamphetamine (3.8 mg/kg) did not generalize to cocaine. Tests with 30 mg/kg of procaine produced 40% cocaine appropriate responses. Cocaine is effective also when administered by gavage into the opening of the proventriculus.

5 The use of the drug discrimination technique for studying structure activity relationships of drugs is discussed.

Introduction

Drugs can be established as discriminative stimuli to control different responses in laboratory animals. The presence or absence of the effects of the training drug indicate to the animal which of two alternative responses will be reinforced during a particular training session. Once this drug discrimination has been acquired, the animal reliably performs a response in accordance with the presence or absence of the cue-complex. At this stage, new drugs can be compared with the training drug in order to assess the degree of generalization between compounds. When tested in this manner, responses learned to the training drug will generalize to sufficiently similar drugs but not to pharmacologically dissimilar drugs (Barry, 1974).

In the present study, dose-effect curves were established at different intervals after the administration of cocaine, norcocaine and, two phenyltropane analogues (WIN 35,428 and WIN 35,065-2) in pigeons trained to discriminate between the presence and absence of effects induced by cocaine (5.6 mg/kg). The structural modifications in the WIN compounds (see below) have been found to result in an increased potency and a prolonged duration of action as demonstrated by stimulation of locomotor activity in mice (Clarke, Daum, Gambino, Aceto, Pearl, Levitt, Cumisky & Bogado, 1973; Heikkila, Cabbat, Manzino & Duvoisin, 1979), rotation behaviour in rats (Heikkila *et al.*, 1979) and, changes in schedule-controlled behaviours of monkeys and pigeons (Spealman, Goldberg, Kelleher, Goldberg & Charlton, 1977; Spealman, Goldberg, Kelleher, Morse, Goldberg, Hakansson, Nieforth, and Lazer, 1979) and rats (D'Mello, Goldberg, Goldberg & Stolerman, 1979). However, unlike cocaine, the WIN compounds are weak local anaesthetics (Clarke *et al.*, 1973). Norcocaine, a N-demethylated metabolite of cocaine, has a pharmacological profile similar to cocaine and has been found to show effects ranging from equipotency to three times the potency of cocaine (Misra, Pontani & Mulé 1976b; Borne, Bedford, Buelke, Craig, Hardin, Kibbe & Wilson, 1977; McKenna, Ho & Englert, 1979; Spealman *et al.*, 1979).

To determine the specificity of the behavioural

0007-1188/81/080843-10 $01.00

stimulus control by cocaine, other psychotropic drugs ((+)-amphetamine, apomorphine, morphine, Δ^9-tetrahydrocannabinol (Δ^9-THC), lysergic acid diethylamide (LSD-25)) were also evaluated for their control of responses learned to the cocaine cue. Procaine was included to evaluate a possible role for local anaesthesia in mediating the cueing effects of cocaine. Furthermore, a possible similar mode of action to cocaine's central effects was tested with a high dose of procaine, since being a nitrogenous local anaesthetic (Ritchie, Cohen & Dripps. 1970), procaine can penetrate into the central nervous system (CNS). Like cocaine, procaine can serve as a reinforcer for self-administration behaviour of monkeys (Ford & Balster, 1977; Hammerbeck & Mitchell, 1978; Johansson, 1980). In this study then, drugs are evaluated for their possible cocaine-like stimulus effects, the dependent variable being the choice behaviour of the animals.

Methods

Animals

Five mature, male White Carneaux pigeons were used, three of which were from Palmetto Pigeon Plant (Sumter, S.C., U.S.A.). The remaining two birds had been obtained from Estuna AB (Norrtälje, Sweden). The birds were reduced to about 80% of their weights (452 ± 62.1 g, mean ± s.d.) after having been on a free-access food-regimen for about one month in the laboratory. The 80% weights were maintained between sessions by the food presented during the sessions and by post-session supplemental feedings. Water and oyster shell grit were always available in the home cages. Between experimental sessions, the birds were individually housed in a large colony room (light from 08 h 00 min–20 h 00 min; temp. 20°–22°C; relative humidity 50–55%).

Apparatus

The experimental chamber, adapted after Ferster & Skinner (1957), was sound-attenuated and ventilated. The response keys, 2 cm in diameter and dimly illuminated with white light, were mounted 10 cm apart on the front panel of the chamber, each key about 19 cm above the chamber floor. The opening of the key contacts defined a key-pecking response. The force required to operate a key was about 15 g. The food magazine was located between the response keys, 4 cm above the floor of the chamber. The reinforcer was 4 s access to grain. The key light went off simultaneously with the 4 s operation of the grain magazine and illumination of food by the magazine light. The chamber was illuminated by a dim-light bulb. Conventional relay programming and recording equipment, located in a room adjacent to that of the chamber, were used.

Procedure

Discrimination training The birds were initially trained to respond on either of the two manipulanda to obtain food according to a FR1 schedule of reinforcement. The requirement for obtaining food was thereafter gradually increased until a FR 15 schedule was in operation i.e. the pigeons had to peck the key 15 times in order to produce access to food for 4 s. When injections were given before a session, the inappropriate manipulandum (left or right) for a given training condition was covered with tape during 5 cocaine sessions and 5 saline sessions, after which the free-choice discrimination training began with both manipulanda available. The birds had to respond on the appropriate manipulandum to produce food. Which manipulandum was correct depended upon whether cocaine or vehicle had been administered prior to the start of the session. Responses on the inappropriate manipulandum had no programmed consequences. The sequential order of drug and non-drug discrimination training sessions was similar to the protocol outlined by Colpaert, Niemegeers & Janssen (1977). The animals were trained once a day 5 days a week for 20 min per session or until grain had been presented 52 times, whichever occurred first. The pigeons were placed in the chamber immediately after the injection and remained there until the training session started 15 min later. The activation of the house light and illumination of the response keys signalled the start of a session. The masking noise and exhaust fan were always in operation.

Testing After the birds had selected the correct key (left or right) at the onset of each training session during at least 8 out of 10 consecutive training days, the animals were changed from the training procedure to the test procedure. Unlike the regular training, both keys were activated throughout a test session and the birds were rewarded for pecking on either of the keys. As in training, food was presented according to a FR-15 schedule of reinforcement i.e. 15 pecking responses had to be accumulated on one of the two keys to produce food. During testing, six rewards could be obtained. Animals were usually tested three times during each two week period. Hence each test was preceded by at least one cocaine and one saline training session. The birds were placed in the chamber immediately after the injection and remained there until the session started when intervals of 15 min or less were tested. Otherwise the pigeons were returned to their home-cages after the injection and were left there until 15 min remained of

the injection-test interval at which time they were placed in the experimental chamber; 15 min later the test session started. The order of tests was mixed. After complete dose-effect gradients had been established for cocaine, norcocaine and the WIN compounds, selected doses were re-evaluated. Also the data listed in Table 3 (see below) involved replications of some of the test conditions.

It may be noted that the present test procedure differs from the test protocol used by Colpaert *et al.* (1977; 1978; 1979) primarily because both keys were open throughout the test session and responses were reinforced on either key regardless of the previous reward.

Drugs

Cocaine hydrochloride, morphine hydrochloride, (+)-amphetamine sulphate (ACO, Sweden), apomorphine hydrochloride, lysergic acid diethylamide tartrate (LSD-25, Sandoz, Switzerland), *para*-hydroxyamphetamine hydrobromide (Smith, Kline & French, U.S.A.), procaine hydrochloride (Sigma) as well as the 1,5-naphtalenedisulphonate salts of the WIN compounds (35,428 and 35,065-2) were dissolved in saline (0.9% w/v NaCl solution) shortly before administration. The WIN compounds were a gift from Sterling-Winthrop Research Institute (Rensselaer, N.Y., U.S.A.). Norcocaine base (N.Y. State Office of Drug Abuse Services, U.S.A.) was dissolved in phosphate buffer (pH 6–7) and thereafter diluted with saline to the desired concentrations. Freshly prepared suspensions of (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC, UN Narcotics Lab., Switzerland) contained 10% propylene glycol, 1% Tween-80 and, saline. The suspensions were prepared as described elsewhere (Sofia, Kubena & Barry, 1974). Intramuscular (i.m.) injections were given in a constant volume (1 ml/kg) in the breast muscle. However, when cocaine and saline were administered by gavage at the opening of the proventriculus with a stainless steel animal-feeding tube, the volume was 2 ml/kg. Doses refer to the forms indicated. The chemical structures of cocaine, norcocaine and, the WIN compounds are illustrated in Figure 1. Expressed as the base, the doses studied of these compounds were: cocaine, 0.89, 2.68 and 5.00 mg/kg (2.94, 8.83 and 16.50 μmol/kg); norcocaine, 0.30, 1.00 and 3.00 mg/kg (1.04, 3.46 and 10.38 μmol/kg); WIN 35,428, 0.20, and 0.66 and 1.98 mg/kg (0.72, 2.38 and 7.15 μmol/kg); WIN 35,065-2, 0.19, 0.64, 1.93 and 3.60 mg/kg (0.73, 2.47, 7.45 and 13.90 μmol/kg).

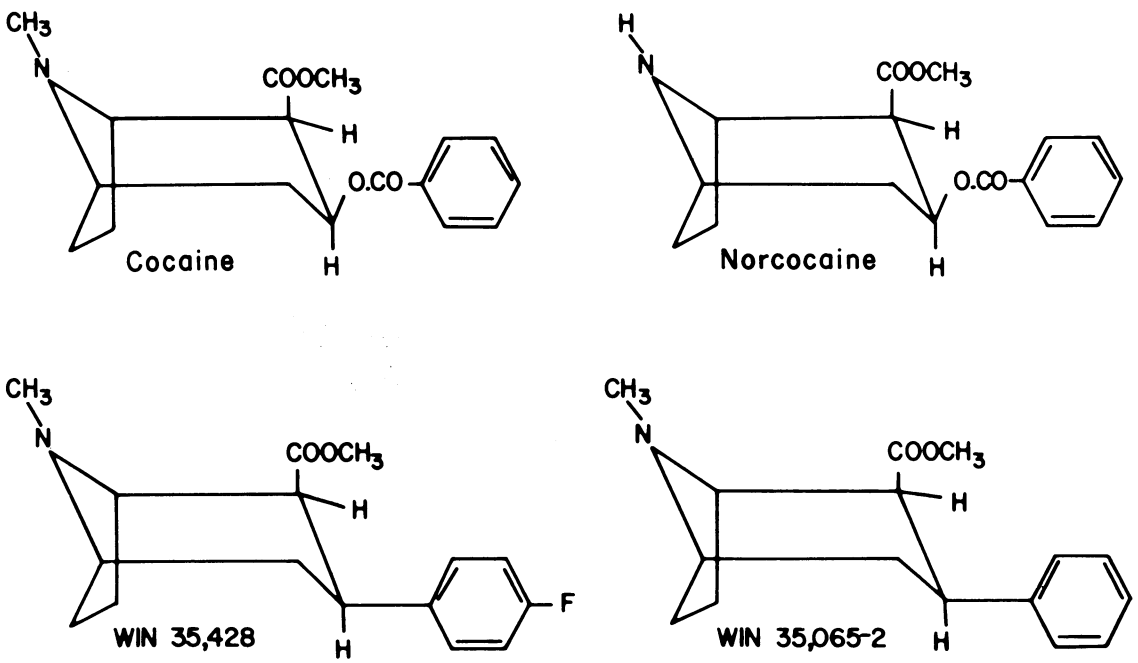

Figure 1 Chemical structures of cocaine, norcocaine, WIN 35,428 and, WIN 35,065-2. In the WIN compounds the esteratic link of cocaine has been eliminated by attaching the benzene ring directly to the tropane skeleton. Also note that WIN 35,428 has a fluorine substitution in the *para* position of the benzene ring. The molecular weights are: cocaine 303; norcocaine 289; WIN 35,065-2 259; and WIN 35,428 277.

Results

Figure 2 shows the results of testing cocaine (dose range 0.0–5.6 mg/kg), norcocaine (dose range 0.0–3.0 mg/kg), WIN 35,428 (dose range 0.0–3.0 mg/kg) and, WIN 35,065-2 (dose range 0.0–5.6 mg/kg) in the five birds trained to discriminate between the presence and absence of cocaine HCl (5.6 mg/kg). Both cocaine and WIN 35,428 were tested at four intervals after injection i.e. 7.5, 15, 60, and 120 min post-injection (p.i.) whereas norcocaine and WIN 35,065-2 were tested 15 and 60 min p.i. The data are presented as the average percentage of responses directed on the drug (cocaine) associated position (% RDP) out of the total number of pecking responses produced during the test session.

The median effective dose estimates, according to the procedure of Litchfield & Wilcoxon (1949), for these generalization gradients are listed in Table 1. The means for some of the data points (i.e. 15 and 60 min p.i.) are based on the average performance of the birds during two different test occasions as shown separately, the second test being conducted after the original dose-effect curve had been determined. The

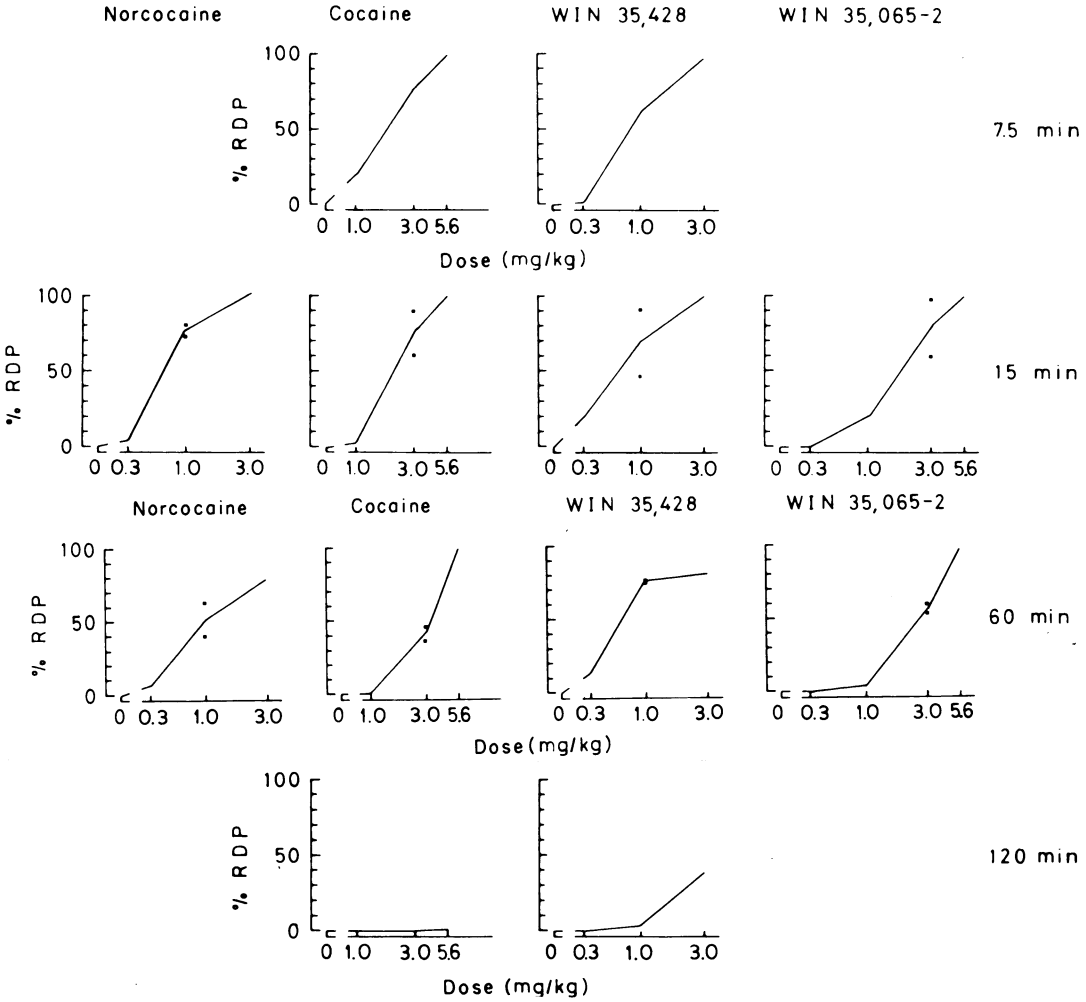

Figure 2 Substitution tests with different doses of cocaine, norcocaine, WIN 35,428 and, WIN 35,065-22 in cocaine-trained pigeons. The pigeons were trained to discriminate between the presence or absence of effects induced by cocaine HCl 5.6 mg/kg. Abscissae, dose in mg per kg. Ordinates, % responses on drug (cocaine) appropriate position (% RDP). Doses as the salts (cocaine and the WIN compounds) or the base (norcocaine). All curves are based on the mean results from five pigeons and all data points were determined on separate days. The means for some of the data points (i.e. 15 and 60 min p.i.) are based on the average performance of the birds during two different test occasions as shown separately. The birds averaged (±s.e.mean) 99.7 (0.14) % and 0.0 (0.0) % cocaine appropriate responses respectively during the initial 6 reward segment of the regular cocaine and saline training sessions preceding the above shown test data. For further details, see text.

Table 1 ED_{50} values for cocaine, norcocaine and the WIN compounds

		Salt			*Base*		
Drug (dose range)	*Time* (min)	ED_{50} (mg/kg)	*% change*	*Slope*	ED_{50} (mg/kg)	*% change*	*Slope*
Cocaine	7.5	1.85		1.65	1.58		1.71
(1.0–5.6 mg/kg)	15	2.10	+13	1.49	1.92	+22	1.45
	60	3.00	+43	1.39	2.68	+40	1.40
	120	>5.60			>5.00		
Norcocaine	15				0.72		1.67
(0.3–3.0 mg/kg)	60				1.15	+60	2.22
WIN 35,428	7.5	0.98		1.71	0.64		1.80
(0.3–3.0 mg/kg)	15	0.66	−34	1.87	0.40	−37	1.98
	60	0.75	+14	2.45	0.48	+20	2.69
	120	>3.00			>1.98		
WIN 35,065-2	15	1.72		1.83	1.15		1.74
(0.3–5.6 mg/kg)	60	2.65	+53	1.57	1.60	+39	1.60

Median effective dose (ED_{50}) estimates for cocaine, norcocaine, WIN 35,428 and, WIN 35,065-2 at various intervals after injection. The estimates are based on the % RDP data shown in figure 2. Doses were plotted both as total amount (salt plus base) as well as the free base only. Note that norcocaine was injected as the free base. % change refers to the percentage difference between adjacent pairs of ED_{50} values. The base constitutes 89.3%, 65.9% and, 64.3% of the total amount (salt plus base) in cocaine, WIN 35,428 and, WIN 35, 065-2 respectively. Slope function is defined as $\frac{ED_{84}/ED_{50} + ED_{50}/ED_{16}}{2}$ (Litchfield & Wilcoxon, 1949).

doses selected for replication were those closest to yielding 50% RDP in the first determination i.e. 3 mg/kg of cocaine, 1 mg/kg of norcocaine, 1 mg/kg of WIN 35,428 and, 3 mg/kg of WIN 35,065-2. Replications were conducted both at 15 and 60 min p.i. The largest deviations (30% to 40%) occurred with cocaine, WIN 35,428 and, WIN 35,065-2 at tests 15 min after the administrations. Otherwise, the two determinations were in close agreement with each other. The ED_{50} values derived from the dose-effect lines indicated that the order of potency was WIN 35,428 > norcocaine > WIN 35,065-2 > cocaine as shown in Table 2. Within-drug comparisons as indicated in Table 1 would suggest a rapid onset of the stimulus effect (cf. cocaine and WIN 35,428 at 7.5 min p.i.), a relative plateau phase (all drugs at 15 and 60 min p.i.) and, a levelling off during the interval 60 to 120 min p.i. of cocaine appropriate responding (cf. cocaine and WIN 35,428). In comparison to cocaine, WIN 35,428 appears to have a slightly slower onset and a somewhat longer duration of action.

All doses that fully substituted for the training dose of cocaine usually resulted in an increased latency to initiate the first key-pecking response and/or a lowered rate of responding when compared to the corresponding saline sessions. The average times to produce six rewards ± s.e.mean for each of the four drugs at the 15 min injection-test interval were respectively: 0.56 ± 0.13 (cocaine, 5.6 mg/kg), 0.53 ± 0.16 (norcocaine, 3.0 mg/kg), 0.49 ± 0.12 (WIN 35,428, 3.0 mg/kg), and 0.86 ± 0.14 (WIN 35,065-2, 5.6 mg/kg). The data are expressed as the quotient between the most recently preceding saline training session and the following test session. Scores below 1.0 indicate a longer time to produce the six rewards; the time (latency) to initiate the first key-pecking response is included in the above scores.

Table 3 shows the results of testing i.m. administered (+)-amphetamine, *para*-hydroxyamphetamine, apomorphine, morphine, Δ^9-THC, LSD-25, and procaine as well as cocaine, administered by gavage. The dose of 3 mg/kg of (+)-amphetamine produced more than 70% responses on the cocaine-associated key both when tested at 15 and 30 min p.i., the highest value being observed after the longer injection-test interval. At approximately equimolar doses, *para*-hydroxyamphetamine (3.8 mg/kg) resulted in a maximum of 3.3% cocaine appropriate responses at the test conducted 30 min p.i.

Table 2 Potency comparisons with cocaine

	Cocaine	
	15 min	*60 min*
Cocaine	1.00	1.00
Norcocaine	2.67	2.33
WIN 35,428	4.80	5.58
WIN 35,065-2	1.67	1.68

ED_{50} values, calculated as the free base, are listed in Table 1.

Tests with apomorphine (0.3–1 mg/kg) resulted in a maximum of 21% appropriate responses at the dose of 0.3 mg/kg. The number of responding animals was reduced in a dose-related fashion and the latency to initiate pecking on the response-keys was increased as compared to the saline training sessions. The birds were observed to peck vigorously at the chamber walls and the floor in a rather stereotyped manner during the apomorphine tests before pecking was directed towards the response keys. A maximum of 17% cocaine appropriate responses were observed during tests with Δ^9-THC (0.3 and 0.56 mg/kg) and morphine (3 and 5.6 mg/kg). Tests with LSD-25 (0.1 mg/kg) and procaine (30 mg/kg) resulted in a higher proportion of cocaine responses but the effect was less than 40%. This dose of procaine caused apparent bleeding and abscesses developed at the site of injection. Thirty min after 10 mg/kg of cocaine, administered by gavage, 82% of the responses were on the cocaine associated key whereas a similar test with saline (2 ml/kg), induced responding appropriate for the nondrug training condition.

Throughout the test periods (i.e. excluding retraining after periods of no training) the five birds performed 100%, 98.7%, 98.4%, 95.7% and, 95.2% (mean ± s.e.mean: 97.6 + 1.03%) correct selections respectively during the cocaine maintenance sessions and the corresponding values for the nondrug, saline sessions were respectively: 98.6%, 98.7%, 100%, 100%, 100% (mean ± s.e.mean: 99.5 ± 0.37%) correct selections. The time needed to complete a training session generally was longer for the cocaine sessions as compared to the saline sessions. Expressed as a proportion between adjacent pairs of saline and cocaine training sessions, the quotients for the five birds were respectively (mean ± s.e.mean): 0.94 (± 0.02), 0.72 (± 0.02), 0.64 (± 0.02), 0.80 (± 0.04) and 0.90 (± 0.01). Data refer to 10 randomly selected comparisons (20 training sessions of 52 rewards each) for each bird. The corresponding key-

Table 3 Substitution tests with procaine and other psychotropic drugs as well as intragastrically administered cocaine

Drug	*Dose* (mg/kg)	*Time* (min)	*No. of tests*	*Responders* (%)	*% responses on cocaine side* (% RDP)	*Time level*	*Binomial* P = Q = 0.50
(+)-Amph	1.0	15	4	100	0.0	1.11 (0.04)	$P = 0.125$
	3.0	15	8	100	72.6	0.78 (0.05)	$P = 0.070$
(+)-Amph	1.0	30	4	100	0.0	1.13 (0.03)	$P = 0.125$
	3.0	30	8	100	85.0	0.68 (0.12)	$P = 0.016$
p-OH-Amph	3.8	15	4	100	3.3	1.02 (0.10)	$P > 0.200$
p-OH-Amph	3.8	30	4	100	0.0	1.09 (0.05)	$P > 0.200$
Apomorph	0.30	15	8	100	21.1	0.32 (0.12)	$P = 0.070$
	0.56	15	8	75	6.7	0.34 (0.11)	$P = 0.008$
	1.0	15	8	63	3.3	0.28 (0.18)	$P = 0.008$
Morph	3.0	45	8	88	17.1	0.31 (0.08)	$P = 0.008$
	5.6	45	4	50	7.9	0.31 (0.35)	$P = 0.125$
Δ^9-THC	0.30	90	8	75	4.9	0.78 (0.12)	$P = 0.070$
	0.56	90	4	50	16.5	0.16 (0.01)	$P = 0.125$
LSD-25	0.056	15	4	100	0.0	0.89 (0.12)	$P > 0.200$
	0.100	15	8	100	29.6	0.59 (0.13)	$P > 0.200$
Procaine	10.0	15	4	100	0.0	1.15 (0.06)	$P = 0.125$
	30.0	15	8	100	39.5	0.79 (0.06)	$P = 0.008$
Cocaine	10.0	30	10	100	82.3	0.77 (0.05)	$P = 0.110$
Saline	–	30	10	100	0.0	1.06 (0.05)	$P > 0.200$

The pigeons were trained to discriminate between i.m. administered saline and 5.6 mg/kg of cocaine, given 15 min before the training sessions. (+)-Amph = (+)-amphetamine SO_4, *p*-OH-Amph = *para*-hydroxyamphetamine HBr, Apomorph = apomorphine HCl, Morph = morphine HCl, Δ^9-THC = (−)-Δ^9-tetrahydrocannabinol, LSD-25 = lysergic acid diethylamide, Procaine = procaine HCl and cocaine = cocaine HCl. Cocaine (10 mg/kg) and saline (2 ml/kg) were tested twice in all five birds and the solutions were administered by gavage at the opening of the proventriculus. For the remaining tests four of the five pigeons were used and all injections were i.m. % responses on the cocaine key (% RDP) is based upon the performance of the responding birds i.e. those birds that obtained at least one reinforcement during the test sessions. Time level (the time needed to produce six rewards) was evaluated as the quotient between the most recently preceding saline training score and the following test session. Three possibilities were considered for the statistical evaluation: an increase (> 1.0), a decrease (< 1.0) or, no change (1.0). The *P* values listed were derived from the Binomial distribution (P = Q = 1/2) and represent the two-tailed probabilities. The pigeons averaged 99.3% and 0.1% cocaine appropriate responses respectively during the first six reward segments of the regular cocaine and saline training sessions preceding the tests listed above.

pecking rates during the saline sessions were respectively (mean ± s.e.mean): 1.24 (±0.02), 1.14 (±0.02), 1.12 (±0.02), 1.18 (±0.02) and 1.44 (±0.01) responses per second.

Discussion

Pigeons were trained to disciminate between the presence and absence of cocaine by responding on one of two keys for food, depending upon whether cocaine (5.6 mg/kg) or saline had been administered. In birds so trained norcocaine, WIN 35,065-2, WIN 35,428, (+)-amphetamine as well as cocaine administered by gavage, could all substitute for cocaine, suggesting that the stimulus properties of these drugs share elements with those of cocaine.

Norcocaine, the N-demethylated metabolite of cocaine in rat (e.g. Nayak *et al.*, 1976) dog (Misra, Patel, Alluri, Mulé, Najak, 1976), monkey (Hawks, Kopin, Colburn & Thoa, 1974; Misra, Giri, Patel, Alluri & Mulé, 1977) and possibly also in man (Inaba, Stewart & Kalow, 1978), was about 2.5 times more potent than cocaine. The potency relationships were fairly similar at both the 15 and 60 min tests indicating only slight differences in the rate of decay of the stimulus effects of these compounds during this time interval. In agreement with this, Misra *et al.* (1976) and Mulé, Casella & Misra (1976) concluded that the rate of disappearance from brain was similar for both drugs in rats and dogs. However, Spealman *et al.* (1979) emphasized the very rapid onset of the effects and the brief duration of action of norcocaine when evaluating its behavioural effects in monkeys and pigeons. The decay of the cocaine cue in pigeons is in reasonable accord with previous studies in rats (Järbe, 1978; McKenna & Ho, 1980) and, as in rats (Järbe, 1978), the onset of the effect is rapid.

The potency order of the cocaine analogues in our pigeons was WIN 35,428 > WIN 35,065-2 > cocaine, which is consonant with previously reported rankings (Clarke *et al.*, 1973; Heikkila *et al.*, 1979; Spealman *et al.*, 1977) although the magnitude of the potency differences appears to be related to both the dependent variable and the species used. Since cocaine is extensively metabolized, primarily by hydrolysis of the ester groups, to more polar metabolites *in vivo* (Archer & Hawks, 1976; Nayak, Misra & Mulé, 1976) it has been suggested (Spealman *et al.*, 1977; 1979) that the metabolism of the WIN compounds, where the benzene ring is attached directly to the tropane configuration, may be slowed down because of the elimination of the esteratic link and as a consequence, may result in a more prolonged duration of action.

Although our data do not refute this hypothesis in general, it is clear that the slope of the generalization gradients were fairly similar at the tests conducted 15 and 60 min after administration indicating no marked differences among the drugs in the decay of the cocaine-like cue effects in pigeons. However, a slightly slower onset of effect is perhaps indicated for WIN 35,428 as compared to cocaine. The structural modifications of the WIN compounds intensified the cocaine-like properties in pigeons (cf. Table 2). The addition of the fluorine substitution at the benzene ring as in WIN 35,428 appears more influential than the elimination of the esteratic link. The potency of norcocaine, in which the esteratic link is retained, was intermediate to that of the WIN compounds. The latter observation suggests a role also for the methyl group on the nitrogen in the structure-activity relationship (SAR) of phenyltropanes. Clarke *et al.* (1973) found that replacement of the N-methyl group by a hydrogen atom enhanced locomotor activity in mice to a level about four times that of WIN 35,065-2. On the other hand, Spealman *et al.* (1979) noted a decline in potency for the N-methylated WIN compound; its activity was intermediate to that of WIN 35,065-2 and cocaine. It would appear that different behaviours are differentially sensitive to chemical changes in the molecule.

How can the drug discimination data be reconciled with earlier studies disclosing a very pronounced locomotor stimulation in mice (Clarke *et al.*, 1973; Heikkila *et al.*, 1979), an enhancement of rotational behaviour in 6-hyroxydopamine lesioned rats (Heikkila *et al.*, 1979), and a greater potency and a longer duration of action of the WIN compounds as compared to cocaine in affecting schedule-maintained behaviours in monkeys and pigeons (Spealman *et al.*, 1977, 1979) and rats (D'Mello, Goldberg, Goldberg & Stolerman, 1979)? The seeming paradox may be more apparent than real. In the drug discrimination procedure the animal has been conditioned to respond differentially to the presence or absence of certain effects of the training drug and it is against this drug dimension (or cue-complex) that new drugs are evaluated. Hence, responses will generalize from the training drug only to the extent that the test drugs mimic the effects of the training cue. It seems possible therefore that the cue-complexes of cocaine and the WIN compounds do not match each other entirely. In support of this, the WIN compounds were very potent inhibitors of re-uptake of tritiated dopamine in the neostriatum of rats (Heikkila *et al.*, 1979) whereas cocaine was considerably less potent in this preparation (Heikkila, Orlansky & Cohen, 1975), and norcocaine has a profile similar to cocaine in this respect (Williams, Clouet, Misra & Mulé, 1977). Furthermore a relatively high dose of cocaine (20 mg/kg) did not induce ipsilateral rotation to a degree comparable to that of the WIN compounds (Heikkila *et al.*, 1979) again implicating differences in the mode of action of the drugs.

The greater tissue affinity of norcocaine over cocaine (Mulé *et al.*, 1976) and its lipophilic character probably explains the potency of norcocaine. The peak concentrations of norcocaine in brain and plasma were approximately 2.3 times those of cocaine (Misra *et al.*, 1976), a value which corresponds well with the potency difference of these compounds seen in the present study. However, McKenna *et al.* (1979) showed that norcocaine HCl was equipotent to cocaine HCl when tested 15 min after intraperitoneal injections in rats trained to discriminate between the presence or absence of 10 mg/kg of cocaine.

That the majority of responses by the pigeons were directed to the cocaine-associated key during tests with (+)-amphetamine (3 mg/kg) is consonant with previous drug discrimination studies in rats (D'Mello & Stolerman, 1977; Ho & McKenna, 1978; Colpaert *et al.*, 1978; 1979) although a relatively higher dose of (+)-amphetamine was required to produce the generalization in this study. A central mediation of this generalization is proposed because of the lack of substitution with *para*-hydroxyamphetamine, a homologue producing effects similar to (+)-amphetamine in the periphery but which, because of its polar character, does not easily penetrate into the CNS (Innes & Nickerson, 1970). It has been concluded previously that the cocaine discrimination is of central origin in rats (Colpaert *et al.*, 1979; McKenna & Ho, 1980). A maximum of 21% cocaine appropriate responses were observed with apomorphine (0.3 mg/kg) in this study. Thus at the doses tested the two drugs produced different effects in the pigeons. Responses learned to cocaine generalized at least partially to the dopamine agonist in rats, but only in doses that suppressed the rate of responding (Ho & McKenna, 1978; Colpaert *et al.*, 1979). Apart from the difference in the species used, there are other procedural variations (dosages, route of administrations) that might account for the discrepancy in the results. Since the generalization was only partial in rats there probably are differences in the stimulus effects of cocaine and apomorphine also in rodents. As expected, morphine, Δ^9-THC, and LSD-25 did not substitute for cocaine.

The lower dose of procaine (10 mg/kg) induced no responses on the cocaine key suggesting that local anaesthesia did not contribute significantly to the cueing effects of cocaine. Also, the WIN compounds are rather weak local anaesthetics (Clarke *et al.*, 1973) thus supporting this conclusion. The 40% cocaine appropriate responses noted during tests with 30 mg/kg of procaine, a behaviourally effective dose in pigeons (McMillan, Dearstyne & Engstrom, 1976), might indicate some common mode of action with cocaine. Similar results were recently observed in rats (McKenna & Ho, 1980).

The 82% cocaine responses seen after cocaine (10 mg/kg) had been administered by gavage into the proventriculus suggest, contrary to earlier beliefs (eg. Ritchie *et al.*, 1970), that measurable amounts of cocaine are taken up from the stomach or the small intestine and can reach the systemic circulation. Recent data in human subjects corroborate these results (eg. Javaid, Fischman, Schuster, Dekirmenjian & Davis, 1978; Van Dyke, Jatlow, Ungerer, Barash & Byck, 1978). This supports the conclusion that generalization between drug stimuli is independent of the route of administration, given that sufficient amounts of the drug reach and can interact with the recognition site. Taken together, the bulk of data clearly indicate pharmacological specificity because only agents closely related to the training drug selectively resulted in a complete substitution.

Because the WIN compounds have been claimed to be both more potent and to have a longer duration of action than the parent compound cocaine, these two agents represented examples where two possible SAR features (potency and duration) were combined in the same chemical configuration. The hypothesis (Spealman *et al.*, 1977; 1979) that the elimination of the esteratic link would lead to a more prolonged duration of action of cocaine-like effects was not borne out. Norcocaine, a compound having an esteratic link, was more potent than WIN 35,065-2 at both the 15 and 60 min injection-test intervals in our pigeons. The extra fluorine moiety, as in WIN 35,428, substantially enhanced the cocaine-like stimulus effects. An extension of this work would be to test also an esteratic compound with a fluorine substitution.

I wish to thank B. Åman for his help in supplying the drugs used and R.L. Clarke, T. Lewander, T.W. Robbins, P.-O. Sjödén and M.D.B. Swedberg for helpful comments on earlier drafts of this manuscript. The technical assistance of G. Ohlin is also acknowledged. The following drugs were generously donated by the manufacturers: apomorphine and LSD (Drs H. Friedly and H. Weidman at Sandoz, Switzerland), norcocaine (Dr A. L. Mistra at New York State Office of Drug Abuse Services, U.S.A.), WIN 35,428 and WIN 35,065-2 (Dr R.L. Clarke at Sterling-Winthrop Research Inst., U.S.A.), Δ^9-THC (Drs O. Braenden and E. Lumsden at U.N. Narcotics Lab., Switzerland) and, *para*-OH-amphetamine from Smith, Kline and French (U.S.A.). The studies were supported by grants from the Swedish Council for Research in the Humanitities and Social Sciences (DNR 583/79 and 497/80).

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(Received November 11, 1980. Revised April 4, 1981.)