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Differential Effects of Direct and Indirect Dopamine Agonists on Eye Blink Rate in Cynomolgus Monkeys

MARK S. KLEVEN and WOUTER KOEK

Centre de Recherche Pierre Fabre, 17 avenue Jean Moulin, 81106 Castres Cedex, France

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ABSTRACT

Spontaneous eye blink rate was assessed in cynomolgus monkeys treated intramuscularly with the high-efficacy dopamine (DA) agonists, (−)-apomorphine, naxagolide, PD 128,907, 2-(N-phenylethyl-N-propyl)amino-5-hydroxytetralin (±)-PPHT, quinpirole, SKF 81297 and SKF 82958; the low-efficacy DA agonists, (−)-3-PPP, roxindole, SDZ 208–912, SKF 75670 and terguride; and the indirect DA agonists, *d*-amphetamine, cocaine, GBR 12935 and methylphenidate. All of the direct DA agonists, with the exception of the partial agonists SDZ 208–912 and terguride, produced significant, dose-related elevations in blink rate. In contrast, none of the indirect agonists increased blink rates when administered over a relatively wide, behaviorally active dose range. These differences suggest either that indirect agonists do not interact with mechanisms involved in eye blinking, or that they have other effects which prevent blink-rate increases. The latter does not appear to be the case because cocaine failed to alter the blink rate-increasing effects of the D_1 agonist, SKF 81297, which suggests that indirect agonists do not mask their own ability to induce blinking. Overall, the results further characterize the involvement of DA receptors in the mediation of spontaneous eye blinks, reveal differential effects of direct and indirect agonists and suggest new directions for research into the neuroanatomical basis of DA-mediated spontaneous eye blinks.

Spontaneous eye blinks, which occur at a rate in excess of that needed to maintain the corneal tear film, are thought to reflect central dopaminergic functioning (Karson, 1983, 1989). This hypothesis is supported by 1) clinical observations in patients with DA-related dysfunctions, *e.g.*, increased blinking in schizophrenia (Karson *et al.*, 1981; Karson, 1983; Ferrier *et al.*, 1984; Kitamura *et al.*, 1984; Lieberman *et al.*, 1987; Klein *et al.*, 1993) and reduced blinking in Parkinson's disease (Karson *et al.*, 1984), and 2) pharmacological studies in both man and nonhuman primates (Karson, 1983; Blin *et al.*, 1990; Peacock *et al.*, 1990; Colpaert *et al.*, 1991; Elsworth *et al.*, 1991; Lawrence *et al.*, 1991) showing that direct DA agonists increase, whereas DA antagonists decrease blink rates in primates (Elsworth *et al.*, 1991; Lawrence and Redmond, 1991; Lawrence *et al.*, 1991) and in humans (Karson, 1983). Thus, both clinical and experimental findings indicate that dopaminergic mechanisms mediate spontaneous eye blinks. Although the brain regions involved have not yet been determined, on the basis of decreased rates of blinking in Parkinsonian patients (Karson *et al.*, 1982; Karson, 1983) and monkeys treated with MPTP (Colpaert *et al.*, 1991; Lawrence and Redmond, 1991), it is likely that spontaneous eye blinks are regulated by mesostriatal pathways. Taken together, the evidence suggests that spontaneous blink rate may offer a useful, noninvasive, *in vivo* measure of DA functioning in humans and in nonhuman primates.

Pharmacological studies in primates indicate that both D_1 and D_2 DA receptor subtypes are involved in blinking (Elsworth *et al.*, 1991). Selective D_1 or D_2 agonists increase blink rates and this effect can be reversed by antagonists that are relatively selective for their respective receptors. For example, blink-rate increases induced by the D_2-like agonist naxagolide are blocked by the D_2 antagonist remoxipride, whereas the effects of the D_1 agonist dihydrexidrine are blocked by the D_1 antagonist SCH 23390 (Elsworth *et al.*, 1991). Furthermore, spontaneous eye blinks are apparently regulated independently by both D_1 and D_2 receptors (Elsworth *et al.*, 1991), because SCH 23390 does not alter the effects of naxagolide, whereas it blocks the effects of the D_1

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ABBREVIATIONS: DA, dopamine; naxagolide, (+)-4-propyl-9-hydroxynaphthoxazine; (±)-PPHT (also designated N-0434), (±)-2-(N-phenylethyl-N-propyl)amino-5-hydroxytetralin; PD 128,907, (+)-(4aR,10bR)-3,4,4a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano-[4,3-b]-1,4-oxazin-9-ol; (−)-3-PPP (also designated preclamol), *S*(−)-3-(3-hydroxyphenyl)-N-propylpiperidine; EMD-49980 (also designated roxindole), 5-hydroxy-3-[4-(4-phenyl-1,2,3,6-tetrahydropyridin-1-yl)butyl]-1H-indole; SDZ 208–912, {N-[(8-α)-2-chloro-6-methylergoline-8-yl]-2,2-dimethylpropanamide}; SKF 81297, (±)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; SKF 82958, (±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; SKF 75670, [*R*,*S*-7,8-dihydro-1H-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine]; GBR 12935, 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)-piperazine; MPTP, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; ANOVA, analysis of variance.

agonist, dihydrexidine (Elsworth *et al.*, 1991). This finding is in contrast to results in a variety of other paradigms in rats and primates in which effects of either direct D_2 agonists and indirect DA agonists are blocked by D_1 receptor antagonists (Mailman *et al.*, 1984; Waddington, 1986; Walters *et al.*, 1987; Kleven *et al.*, 1990). Apart from studies demonstrating the involvement of D_1 and D_2 receptor subtypes, there are no systematic studies of the effects of different classes of dopaminergic agonists on blink rate.

The purpose of the present study was to characterize further the effects of direct D_2-like (*i.e.*, D_2/D_3) and D_1 agonists on eye blinking in the cynomolgus monkey and to compare their effects with those of indirect DA agonists. Because it has been suggested that low-efficacy D_1 agonists are unable to increase blink rate (Elsworth *et al.*, 1991), the effects of D_1 agonists varying in efficacy were examined. Further, to examine the effects of compounds with low D_2 efficacy, the partial agonists (−)-3-PPP, terguride, roxindole and SDZ 208–912 (Seyfried *et al.*, 1989; Arnt and Hyttel, 1990; Coward *et al.*, 1990; Svensson *et al.*, 1991; Bartoszyk *et al.*, 1996) were examined and compared with the effects of higher efficacy D_2-like agonists such as (−)-apomorphine and quinpirole. We report here that, whereas direct DA agonists readily increased spontaneous eye blinks, the indirect DA agonists cocaine, *d*-amphetamine, GBR 12935 and methylphenidate did not have comparable effects, even at doses that were evidently behaviorally active. Further, the finding that cocaine did not alter the blink rate-increasing effects of the D_1 agonist, SKF 81297, suggests that indirect agonists may be unable to increase DA activity at the site(s) that mediate the blink rate-increasing effects of direct DA agonists.

Methods

Animals. Four young adult female cynomolgus (*Macaca fasicularis*) monkeys weighting from 4.9 to 6.4 kg were obtained from Charles River France. All of the monkeys were drug naive at the start of the present experiments; however, testing of DA agonists was interrupted by several periods during which animals received low doses of neuroleptics alone or in combination with (−)-apomorphine. Animals were housed individually in stainless steel cages (floor area: 1 m^2) soon after arrival and were habituated to intramuscular injections and to the observation procedure (see below) over a period of approximately 2 months before the acute administration of drugs used in the present study. Animals were housed in an air-conditioned and controlled room (21 ± 1°C and relative humidity 55 ± 5%) under a 12 h:12 h light:dark cycle (lights on at 7:00 A.M.) with filtered (0.2 μ) water continuously available. Animals were fed standard monkey chow (Singe Croquettes: Extralabo, Provins, France), supplemented by fresh fruit daily and vitamins (Prima-Treats: BioServ; Frenchtown, NJ). Monkeys were handled and cared for in accordance with guidelines set by the U.S. Department of Health and Human Services for humane treatment of animals (Guide for the Care and Use of Laboratory Animals, U.S. DHHS, PHS, NIH publication 85–23, revised 1985) and the protocol (No. 138) was carried out in compliance with local ethical committee guidelines for animal research.

Behavioral procedure. The measurement of blinks was essentially as described previously (Elsworth *et al.*, 1991; Lawrence and Redmond, 1991). A blink was defined as a closure of the eyelids lasting no longer than approximately 0.5 sec as judged by the observer. Animals were observed in their home cages during consecutive 2.5-min periods, and the number of blinks and the duration of time that the eyes were visible to the observer were recorded during each period (if this duration was less than 1.5 min, the eye blink counts for that period were not used in subsequent analyses).

Animals were observed at 12.5-min intervals during three epochs: 1) base line (−90 to −60 min), consisting of three observation periods before injection of saline or vehicle; 2) control (−30 to 0 min), consisting of two periods after the injection of saline or vehicle; 3) treated (0–62.5 min), consisting of five periods after the injection of agonists or vehicle. Each of the four animals was observed twice weekly (Tuesday and Thursday or Monday and Wednesday) between 2:00 and 5:00 P.M.. During each session, some animals were treated with drug, and others with vehicle, and a control session, during which saline or vehicle was injected at both the −45 and 0 min time points, was conducted at least once every four sessions.

On the basis of observations of blink rates during the adaptation period before drug testing began, criteria for testability were implemented to ensure that within-session variability was minimal during test sessions. Thus, testing took place only when blink rates during each of the observation periods of the preceding control session deviated less than ± 2 S.D. from the mean of the three initial control periods (base line). Control sessions were continued until the criterion for testability was met. Because control sessions were conducted a minimum of once every four sessions, this allowed frequent assessment of the stability of eye blink rates within a session.

Data analysis. Each session generated data on two variables: the number of blinks and the time that blinks could be observed, during each 2.5-min period. Drug effects on blink rate were evaluated for significance by: 1) a two-factor ANOVA (dose *vs.* period) on data obtained during the entire session, with *post hoc* comparisons made among doses with Duncan's New Multiple Range test, and 2) a two-factor ANOVA of data obtained immediately before and after agonist treatment, followed by contrasts comparing the pooled control with each of the dose groups, with the per experiment level of significance corrected for multiple comparisons (Winer, 1971) and a $P < .05$ deemed statistically significant. Maximal effects were compared by a one-factor ANOVA of only data from the doses of each drug which produced the highest mean increase in blink rate and *post hoc* comparisons made among the drugs by use of the Newman Keuls test. Although most of the drugs were tested with four monkeys per dose, because of the death of one of the monkeys in another experiment, some of the drugs and/or doses were not completed in all four monkeys.

Comparisons between potency estimates and binding affinities at D_2 and D_3 receptors were made by the method described by Bergman *et al.* 1995. The ED_{50} (defined as the dose which increased blink rates to 50% of the maximum) values were determined by linear interpolation of the ascending portion of the dose-response functions, and values of individual monkeys were averaged for each drug. Binding affinities relative to (−)-apomorphine, with the exception of roxindole, were taken from published radioligand-binding experiments in which (−)-apomorphine and at least four other D_2-like agonists were also studied (Madras *et al.*, 1988; Seeman and Van Tol, 1993; Chio *et al.*, 1994; Freedman *et al.*, 1994; Sautel *et al.*, 1995). Because binding studies directly comparing roxindole and (−)-apomorphine were not available, the relative affinity of roxindole was derived with published results for (−)-apomorphine and D_2 and D_3 affinities from Bartoszyk *et al.* (1996).

Drugs. The compounds used were (−)-apomorphine HCl (MW 304), GBR 12935 HCl, (−)-quinpirole HCl (MW 256), PD 128,907 HCl (MW 286), (−)-3-PPP HCl (MW 256), (±)-PPHT HCl (MW 346), SKF 81297 HCl (MW 371; (±)-6-chloro-PB, SKF 82958 HCl (MW 411; chloro-APB), terguride maleate (all from Research Biochemicals Intl., Natick, MA), *d*-amphetamine HCl (Calaire Chimie, Calais, France), cocaine HCl (coopération pharmaceutique française, Melun, France), methylphenidate HCl (Ciba-Geigy Co., Basle, Switzerland), naxagolide (MW 247; Merck Sharp & Dohme Research Laboratories, Rahway, NJ), SKF 75670 (MW 350; Smith Kline Beecham, King of Prussia, PA), roxindole mesylate (MW 443; EMD 49980; E. Merck, Darmstadt, Germany) and SDZ 208–912 mesylate (Sandoz Pharma

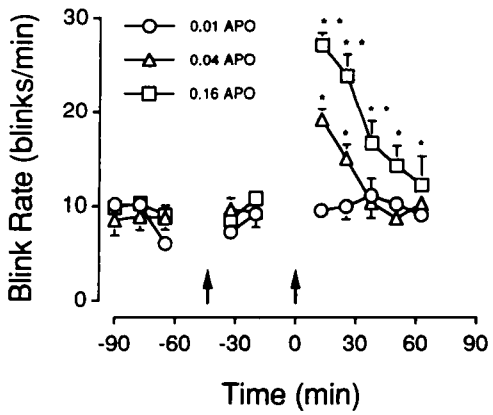

Fig. 1. Time course of effects of the direct DA agonist, (−)-apomorphine (APO; 0.01–0.16 mg/kg i.m.) on spontaneous blink rate in cynomolgus monkeys. Values represent the mean ± S.E.M. blinks/min (n = 4/dose). Arrows indicate the injection times of injection of saline (T-45) and drug (T-0). Where absent, error bars are contained by the symbols. *P < .05 *vs.* the lowest dose, 0.01 mg/kg, **P < .05 *vs.* the intermediate dose, 0.04 mg/kg.

AG, Basle, Switzerland). Drugs, with the exception of GBR 12935, (±)-PPHT, terguride, SKF 75760, roxindole and SDZ 208,912, which were prepared as suspensions in aqueous Tween 80 (2 drops/10 ml distilled water), were dissolved in distilled water and injected i.m. in a volume of 1 ml/10 kg. Doses of all drugs refer to the free base.

Results

Under control conditions, eye blinks occurred at mean rates ranging from approximately 7 to 10 blinks/min and were relatively stable after administration of saline (*i.e.*, the within-session coefficient of variation usually ranged between 10 and 20%). Observation times during control sessions ranged from 110 to 120 sec and there were no systematic differences among the 10 observation periods comprising a session. Overall, data were excluded from fewer than 10% of total observation periods as a result of observation times smaller than the criterion 90 sec.

Effects of D_2-like agonists. Administration of direct DA agonists produced significant, dose- and time-related increases in spontaneous blink rate as represented in figure 1 by the results obtained with the nonselective DA agonist, (−)-apomorphine. Significant increases in blink rate were generally found, as in the case of (−)-apomorphine, during the first two observation periods after drug administration, and diminished as a function of time thereafter. Data from the first two periods after drug administration were analyzed separately and averaged for each of the direct-acting DA agonists and are shown in figure 2. None of the direct agonists had systematic effects on observation times (data not shown).

Average blink rates increased in a dose-related manner after administration of all of the DA agonists shown in figure 2A. There were few apparent differences in potency, with the exception that naxagolide was more potent than the other compounds in that lower doses produced significant increases in eye blinks (table 1) and the upper 95% confidence limit of its ED_{50} was lower than the ED_{50} values of the remaining compounds (table 2). In general, the lowest doses of the direct agonists did not significantly increase blink rate in comparison with rates observed during the control periods when saline was injected, whereas significant increases were found after administration of intermediate and high doses (table 1). Differences in maximal blink-rate increases were also apparent in that (−)-apomorphine and quinpirole produced increases in blink rate which were significantly higher (P < .05) than the maximal effects produced by naxagolide, (±)-PPHT or PD 128,907.

Effects of D_2-like partial agonists. As shown in figure 2B, the partial DA agonist (−)-3-PPP produced significant dose-related increases in blink rate, with a maximal effect similar to that produced by the 0.16 mg/kg dose of (−)-apomorphine. In contrast, the partial agonist, roxindole produced maximal effects which were significantly lower than those of (−)-apomorphine, but not (−)-3-PPP, whereas the remaining compounds, terguride and SDZ 208–912, were not effective even when tested at doses up to 2.5 mg/kg. The highest dose of SDZ 208–912 was tested in only two monkeys because it produced brief sedation and subsequent lethargy, which lasted for several days after the injection.

The comparison of ED_{50} values of D_2-like agonists to increase blink rates (table 2) and results from previously published binding studies (fig. 3) revealed a significant positive correlation between D_2 receptor binding affinities relative to (−)-apomorphine (Madras *et al.*, 1988; Chio *et al.*, 1994; Freedman *et al.*, 1994; Sautel *et al.*, 1995) and the behavioral

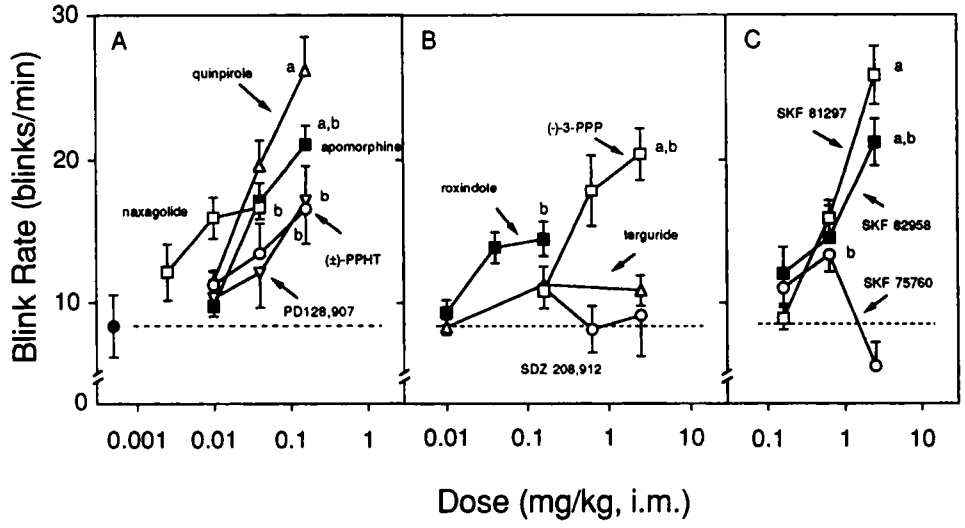

Fig. 2. Effects of DA agonists on spontaneous blink rate in cynomolgus monkeys. Values are the mean ± S.E.M., blinks/min (n = 3–4/dose, with the exception of 2.5 mg/kg SDZ 208–912 and SKF 75670, n = 2), and represent data obtained during two, 2.5-min observation periods starting 12.5 and 25 min after drug administration. (A) D_2-like agonists; (B) D_2-like partial agonists; (C) D_1 agonists. The closed circle and dashed line represent the mean blink rate observed during the control period before drug injection. Different letters above maximal effects of individual drugs indicate that their maximal effects differed significantly from other groups that do not share the same letters (P < .05). See table 1 for doses with significant effects on blink rate.

TABLE 1
Doses of direct and indirect DA agonists with significant effects on blink rates in cynomolgus monkeys

Drug	Base Line Blinks/min	Dose	Drug Treated Blinks/min	P
(−)-Apomorphine	9.5 ± 0.9	0.04	17.1 ± 1.2	.0003
	9.0 ± 0.7	0.16	21.1 ± 1.3	.0003
Quinpirole	7.5 ± 0.7	0.01	11.4 ± 0.8	.03
	8.7 ± 0.5	0.04	19.6 ± 1.8	.0003
	7.6 ± 0.6	0.16	26.2 ± 2.3	.0003
Naxagolide	8.6 ± 0.5	0.0025	12.1 ± 1.9	.05
	9.0 ± 0.5	0.01	15.9 ± 1.4	.0003
	7.8 ± 0.9	0.04	16.6 ± 1.1	.0003
(±)-PPHT	9.2 ± 1.0	0.04	13.4 ± 2.1	.03
	8.2 ± 0.6	0.16	16.5 ± 2.4	.0003
PD 128,907	8.2 ± 0.7	0.16	17.1 ± 2.5	.0003
(−)-3-PPP	10.2 ± 1.0	0.63	17.8 ± 2.5	.0003
	8.3 ± 0.5	2.5	20.4 ± 1.8	.0003
Roxindole	8.0 ± 0.8	0.04	13.8 ± 1.1	.0003
	8.6 ± 0.6	0.16	14.4 ± 1.2	.0003
SKF 81297	7.0 ± 0.7	0.63	15.8 ± 1.2	.0003
	8.1 ± 0.5	2.5	25.8 ± 2.0	.0003
SKF 82958	8.1 ± 0.6	0.63	14.5 ± 2.3	.0003
	8.0 ± 0.8	2.5	21.2 ± 1.7	.0021
SKF 75670	7.4 ± 0.5	0.16	11.0 ± 1.1	.024
	9.0 ± 0.8	0.63	13.3 ± 1.2	.0012
Cocaine	8.0 ± 0.5	0.63	6.0 ± 0.5	.029
	9.1 ± 0.6	2.5	5.7 ± 1.2	.04

Values are mean ± S.E.M., n = 3 to 4 animals/dose.

TABLE 2
Comparison of ED_{50} values for blink rate-increasing effects of D_2-like and D_1 agonists

Drug	ED_{50}	95% Confidence Limit	Relative Potency
D_2-like agonists	*μmol/kg*		*vs. (−)-apomorphine*
1. Naxagolide	0.011	0.004–0.030	0.11
2. Roxindole	0.056	0.020–0.16	0.56
3. (−)-Apomorphine	0.10	0.055–0.20	1.0
4. (±)-PPHT	0.11	0.032–0.36	1.1
5. Quinpirole	0.14	0.10–0.21	1.4
6. PD128,907	0.23	0.037–1.4	2.3
7. (−)-3-PPP	2.1	0.95–4.6	21
D_1 agonists			*vs. SKF75670*
SKF 75670	0.66	0.34–1.3	1.0
SKF 81297	2.1	1.4–3.3	3.2
SKF 82958	2.7	2.1–3.5	4.1

potencies relative to (−)-apomorphine (r = .87, P < .008). The correlation between relative D_3 receptor affinity (Seeman and Van Tol, 1993; Chio *et al.*, 1994; Freedman *et al.*, 1994; Sautel *et al.*, 1995) and behavioral potencies failed to reach significance (r = .71, P > .07).

Effects of D_1 agonists. Each of the D_1 agonists produced significant, dose-related increases in blink rates (fig. 2C; table 1), with SKF 75670 showing the highest potency relative to SKF 81297 or SKF 82958 (table 2). However, the estimated ED_{50} value of SKF 75760 may not be compared easily with the other D_1 agonists, because a significantly lower maximal effect was obtained after administration of the intermediate dose, 0.63 mg/kg (13.3 ± 1.2 blinks/min) in comparison with the effects of the 2.5 mg/kg dose of SKF 81297 (25.8 ± 2.0) or SKF 82958 (21.2 ± 1.7). The highest dose of SKF 75670 (2.5 mg/kg) did not produce elevations in blink rates. Although insufficient drug was available to test more than two monkeys at this dose, the results suggest that this dose produced rate-decreasing effects.

Effects of indirect agonists. Unlike the results obtained with the direct agonists, none of the indirect agonists caused significant increases in blink rate at any time during the 60-min period during which animals were observed, represented in figure 4 by cocaine. The analysis of data obtained during the first two observation periods after drug administration (fig. 5) revealed significant overall decreases after administration of cocaine and methylphenidate, whereas *d*-amphetamine and GBR 12935 did not significantly alter blink rates. *Post hoc* analysis showed that only administration of the 0.63 and 2.5 mg/kg doses of cocaine produced significant decreases in blink rate (table 1).

Although the indirect DA agonists failed to increase blink rate, they significantly increased the amount of time during which blinks could be observed, not only immediately after drug administration (fig. 6), but also during the remainder of the session. *Post hoc* analysis showed that significant increases occurred immediately after administration of each of the doses of cocaine and the highest doses of methylphenidate, amphetamine and GBR 12935. It is important to note that these effects represent a marked increase in the amount of time that the animals faced the observer. For example, after administration of the 2.5 mg/kg dose of cocaine, monkeys spent almost the entire 150-sec period facing the observer, whereas under control conditions, in general, the eyes

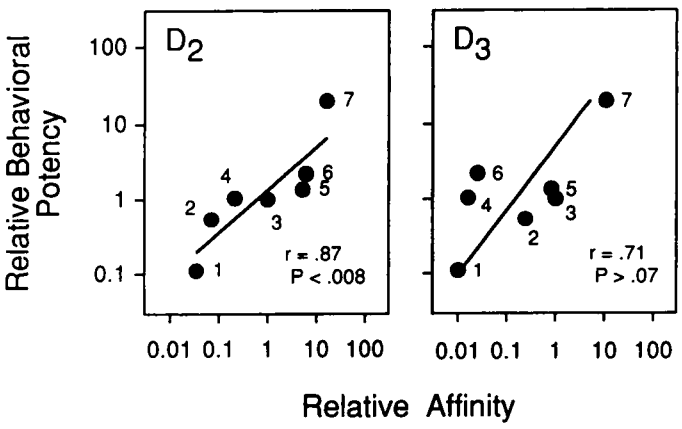

Fig. 3. Relationship between behavioral potency of D_2-like agonists to increase blink rate and their binding affinity for D_2 and D_3 receptors relative to (−)-apomorphine. Numbers refer to data shown in table 2. Relative affinities were obtained from results of published radioligand binding experiments (see "Methods").

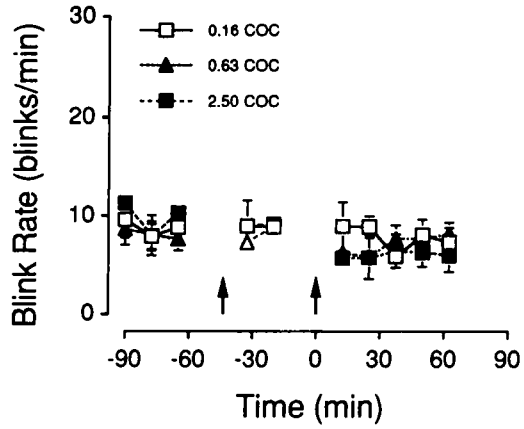

Fig. 4. Time course of effects of the indirect DA agonist, cocaine (COC; 0.16–2.5 mg/kg i.m.) on spontaneous blink rate in cynomolgus monkeys. Values are the mean ± S.E.M. blinks/min (n = 4/dose). Arrows indicate the time of injection of saline (T-45), and drug (T-0). Where absent, error bars are contained by the symbols.

could not be observed for a period of approximately 30 to 35 sec.

Effects of pretreatment with cocaine. Because the differential effects of direct and indirect agonists suggested either that indirect agonists are unable to produce increases or that they produce other effects that mask blink-rate increases, monkeys (n = 3) were treated with saline or cocaine (0.16–2.5 mg/kg; T-45 min) before administration of SKF 81297 (2.5 mg/kg; T-0 min). The results obtained during the entire session are shown in figure 7. Although the highest dose of cocaine appeared to inhibit blinking after SKF 81297 treatment at certain time points, dose-related effects were not consistently obtained over the entire session. Examination of the effects obtained during the periods immediately before and after SKF 81297 treatment (table 3) showed only a small decrease in blink rate after treatment with the 2.5 mg/kg dose of cocaine (19.8 ± 1.8 *vs.* 14.2 ± 2.6, saline *vs.* cocaine, respectively). As in the previous experiment in which cocaine was administered at T-0 min, cocaine pretreatment increased observation times, which, because of high variability, did not reach significance (P > .20); observation times were not different from control after the administration of SKF 81297 (table 2).

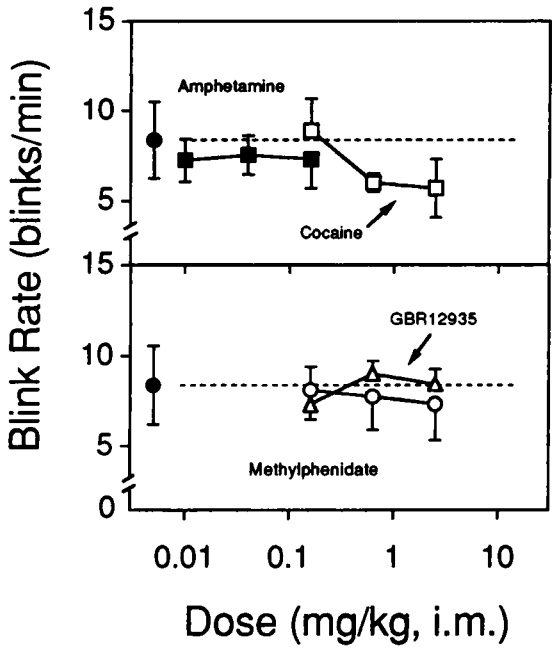

Fig. 5. Effects of indirect DA agonists on spontaneous blink rate in cynomolgus monkeys. Values represent the mean blink rate in four monkeys. Other details are as in figure 2.

Discussion

The most important finding of this study is that direct and indirect DA agonists have differential effects on eye blink rate in cynomolgus monkeys. All of the high-efficacy direct DA agonists reliably produced significant increases in blink rate, confirming and extending previous findings (Karson, 1983; Lewis *et al.*, 1990; Elsworth *et al.*, 1991; Lawrence and Redmond, 1991), whereas a variety of indirect agonists failed to increase blink rate when tested over a wide range of doses. The finding that indirect agonists significantly increased the amount of time that animals faced the observer shows that the failure to obtain eye blink increases after high doses of psychomotor stimulants is not caused by a reduced opportunity to observe the animals. Finally, the rate-increasing effects of a D_1 agonist, SKF 81297, were not significantly reduced by pretreatment with cocaine, which suggests that the absence of blink-rate increases is not due to a general masking effect of the indirect agonists. The present results therefore indicate that indirect and direct DA agonists differ in their ability to increase spontaneous eye blink rate in cynomolgus monkeys.

The effects of direct-acting D_2 agonists in the present study replicate a number of previously reported results and extend the pharmacological characterization to include the high-efficacy, D_2-like agonists (±)-PPHT and PD 128,907. The finding that the nonselective $D_1/D_2/D_3$ agonist, (−)-apomorphine, significantly increased blink rate, with more than a doubling of rate observed at the highest dose tested in the present study (0.16 mg/kg i.m.), is in complete agreement with results reported previously in human and nonhuman primates (*e.g.*, Blin *et al.*, 1990; Lawrence and Redmond, 1991; Migler *et al.*, 1993). The effects obtained in the present study with the relatively selective D_2-like agonists quinpirole

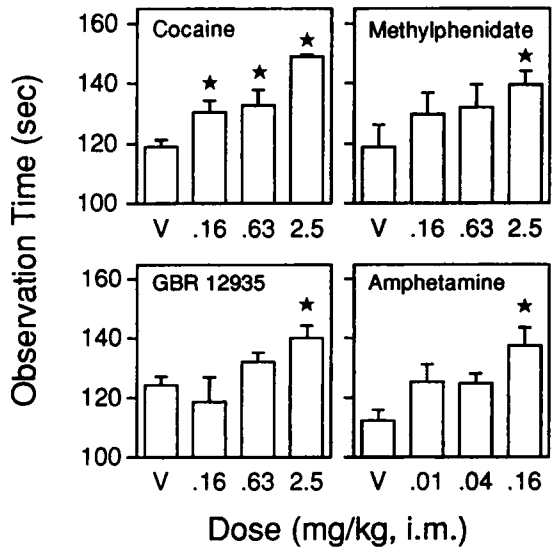

Fig. 6. Effects of indirect DA agonists on observation time in cynomolgus monkeys (n = 3–4). Values are the mean + S.E.M. observation time (sec). V: pooled average of blink rate obtained during the control periods before the injection of agonists. Data following drug treatment represent the average from both periods after drug administration (T12.5 and T25 min). *P < .05 *vs.* control (V).

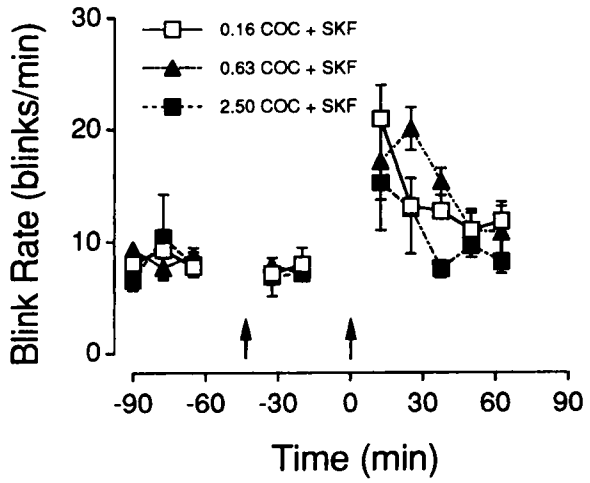

Fig. 7. Time course of effects of SKF 81297 (SKF; 2.5 mg/kg i.m.) in combination with cocaine (COC; 0.16–2.5 mg/kg) on spontaneous blink rate in cynomolgus monkeys. Values are the mean ± S.E.M. blinks/min (n = 3/dose). Arrows indicate the time of injection of cocaine (T-45), and SKF 81297 (T-0). Where absent, error bars are contained by the symbols.

and naxagolide are also consistent with previous findings in monkeys (Peacock *et al.*, 1990; Elsworth *et al.*, 1991). Overall, the concordance between results of the present study and the literature indicates that the effects of direct D_2 agonists on blink rate are highly replicable across different laboratories and primate species.

In this study, the partial D_2-like agonist (−)-3-PPP produced increases in eye blinks comparable to those obtained with the higher efficacy DA agonists (−)-apomorphine and quinpirole, whereas roxindole produced relatively lower effects and terguride and SDZ 208–912 were not effective. The rank order of the magnitude of blink-rate increases obtained with these partial D_2-like agonists, *i.e.*, (−)-3-PPP > roxindole > terguride = SDZ 208–912, is consistent with their reported relative intrinsic activity *in vivo* (Seyfried *et al.*, 1989; Arnt and Hyttel, 1990; Coward *et al.*, 1990; Svensson *et al.*, 1991; Lahti *et al.*, 1992). Although the intrinsic activity of D_2 agonists can vary among assays, terguride and SDZ 208–912 uniformly exhibit lower intrinsic activity relative to (−)-3-PPP. For example, under conditions in which (−)-apomorphine and quinpirole produce significant decreases in rat striatal homovanillic acid levels, (−)-3-PPP is ineffective (Lahti *et al.*, 1992), whereas SDZ 208–912 and terguride produce increases (Coward *et al.*, 1990; Lahti *et al.*, 1992). By also using electrophysiological techniques in rats, (−)-3-PPP reportedly has intrinsic activity of approximately 50% that of (−)-apomorphine, whereas terguride is approximately 25% and SDZ 208–912 is negative (Lahti *et al.*, 1992). Thus, in comparison with agonists with high intrinsic activity, (−)-3-PPP has intermediate activity, whereas terguride and SDZ 208–912 exhibit effects that may be indistinguishable from antagonists like haloperidol. Because (−)-3-PPP was as effective in the present study as (−)-apomorphine, the results indicate that blinking can be produced by compounds showing intermediate to high levels of intrinsic activity. Nonetheless, low-efficacy agonists such as terguride or SDZ 208–912 did not produce effects on eye blink rate, and roxindole, which reportedly also has lower efficacy than (−)-3-PPP (Seyfried *et al.*, 1989) produced both low effects and an inverted U-shaped dose-response function, which suggested that intermediate intrinsic activity may be required for the induction of eye blink increases.

The results obtained with D_1 agonists in this study are also in agreement with previous results obtained in monkeys (Peacock *et al.*, 1990; Elsworth *et al.*, 1991). The present finding that the partial agonist SKF 75670 caused relatively small effects on eye blink rate is consistent with a preliminary report that SKF 38393 does not produce increases in blinking in the monkey (Karson, 1988), whereas SKF 81297 and SKF 82958 (present results; Peacock *et al.*, 1990) and dihydrexidine (Elsworth *et al.*, 1991) produce relatively larger blink-rate increases. With respect to stimulation of adenylyl cyclase in rat and monkey brain tissue, SKF 81297, SKF 82958 and dihydrexidine generally exhibit higher efficacy than SKF 38393 and SKF 75670 (Arnt *et al.*, 1988; O'Boyle *et al.*, 1989; Andersen and Jansen, 1990; Izenwasser and Katz, 1993). Thus, the present findings suggest that relatively high intrinsic activity at D_1 receptors may be needed to produce blink-rate increases (Elsworth *et al.*, 1991).

Together, the results obtained with partial D_2 and D_1 agonists indicate that spontaneous eye blinks are sensitive with respect to intrinsic efficacy of DA agonists; however, it should be noted that naxagolide and (±)-PPHT produced effects of a significantly lower magnitude than quinpirole, whereas they reportedly possess high efficacy (Freedman *et al.*, 1994). Additionally, preliminary results obtained with higher doses of several drugs examined in this study (naxagolide, (−)-apomorphine and PD 128,907) suggested that high-efficacy agonists may exhibit inverted U-shaped dose-response functions. These effects could not be systematically investigated because of behavior-disrupting effects which accompany high doses of stimulants; however, it is conceivable that high intrinsic activity may be incompatible with large increases of blink rate. Further studies will be necessary to determine whether this behavioral assay can resolve differ-

TABLE 3

Pretreatment with cocaine does not alter the effects of the D_1 agonist SKF 81297 (2.5 mg/kg) on blink rate and observation time

Values are mean ± S.E.M. (n = 3 monkeys per group) obtained from the first two periods after saline or SKF 81297 administration (see "Methods" for details).

Cocaine	Blink Rate		Observation Time	
	+Saline	+SKF 81297	+Saline	+SKF 81297
	blinks/sec		*sec*	
Saline	7.9 ± 1.0	19.8 ± 1.8	135 ± 3.5	130 ± 9.6
0.16	7.6 ± 0.7	17.8 ± 2.6	132 ± 7.6	135 ± 8.3
0.63	7.5 ± 0.3	18.6 ± 1.9	149 ± 0.7	122 ± 9.2
2.5	7.0 ± 0.8	14.2 ± 2.7	147 ± 1.7	142 ± 4.0

ences among compounds with intermediate to high efficacy. Nonetheless, because terguride and SDZ 208-912 were inactive, and roxindole and SKF 75670 produced both low maximal effects and inverted U-shaped dose-response functions, this study demonstrates that DA agonists with little intrinsic activity can be distinguished from higher efficacy compounds.

In this study, the D_2-like agonist, PD 128,907 (Akunne *et al.*, 1995; Pugsley *et al.*, 1995; Sautel *et al.*, 1995) produced dose-related increases in eye blinks. Taken together with reports that other compounds examined in this study, such as (−)-3-PPP and the remaining compounds shown in the left panel of figure 2, also have high affinity for the D_3 receptor (Freedman *et al.*, 1994; Sautel *et al.*, 1995), our findings raise the possibility that D_3 receptors may be involved in blinking. Although controversial, it is currently believed that D_3 receptors are primarily located somatodendritically (Waters *et al.*, 1993; Svensson *et al.*, 1994), with a high density in limbic areas of the brain (Gehlert *et al.*, 1992). Pharmacological studies suggest that the D_3 receptor functions as an autoreceptor (Pugsley *et al.*, 1995). Although autoreceptor mediation of eye blinks cannot be ruled out entirely on the basis of the present results, a more important consideration is that, with the possible exception of PD 128,907, *in vivo* preference for D_3 receptors is problematic (see Seabrook *et al.*, 1995). That is, D_2:D_3 selectivity is often based on the comparison of binding to low-affinity D_2 agonist sites with binding to high-affinity D_3 sites, whereas comparisons of functional effects indicate that, of the compounds examined in this study, only PD 128,907 exhibits more than a 2- to 3-fold preference for the D_3 receptor (Pugsley *et al.*, 1995; Sautel *et al.*, 1995). In this respect, quinpirole and (−)-apomorphine can be considered nonselective with respect to D_2:D_3 affinity; findings that these compounds, in addition to the more D_2 selective agonist, bromocriptine (Sautel *et al.*, 1995), increase eye blinks (Karson, 1983) implicate the involvement of D_2 receptors.

The significant correlation between behavioral potencies and D_2 binding affinities relative to (−)-apomorphine offers additional evidence to support the idea that D_2 receptors mediate increases in eye blinks. However, the correlation between D_3 binding and relative behavioral potencies approached significance ($P < .10$), making it difficult to exclude the involvement of D_3 receptors on the basis of the set of compounds examined in this study. Given that high-efficacy D_2 agonists also exhibit high affinity for D_3 receptors (Freedman *et al.*, 1994), a correlational approach to this question may not be sufficient to rule out a possible role for D_3 receptors. Thus, definitive evidence may only be obtained when selective D_3 antagonists become available.

The present results demonstrate that indirect agonists do not have effects on eye blinks that are similar to those obtained with direct DA agonists. It is unlikely that inadequate doses were tested because the indirect and direct DA agonists have behavioral effects in primates at doses similar to those used in the present study (see *e.g.*, Melia and Spealman, 1991; Bergman *et al.*, 1995). Furthermore, the finding that observation times were significantly increased indicates not only that behaviorally active doses were used, but also that the indirect agonists increased the opportunity to detect changes in blink rate. Although to our knowledge these are the first such results in monkeys, methylphenidate has been reported to increase blink rate in schizophrenics either withdrawn from or undergoing neuroleptic treatment (Lieberman *et al.*, 1987). Although species differences are a possible explanation, there is generally good agreement between behavioral effects of psychomotor stimulants in monkeys and humans (see Woods *et al.*, 1987), and there is otherwise agreement between human and monkey eye blink studies (Karson, 1983, 1989). It is possible that the augmented blink rate reported to accompany schizophrenia may facilitate rate-increasing effects of methylphenidate, or, alternatively, prolonged neuroleptic treatment may induce sensitization to the effects of methylphenidate on blink rate. Unfortunately, the effects of methylphenidate or other indirect agonists on blink rate in a nonschizophrenic population do not appear to have been studied. In contrast, the present results clearly demonstrate that indirect dopamine agonists do not increase eye blinks in the monkey.

The differential effects of direct and indirect agonists found in the present study complement several previously reported findings in other behavioral models (Rosenzweig-Lipson *et al.*, 1994; Katz *et al.*, 1995; Tirelli and Witkin, 1995). For example, the present results are similar, albeit opposite in direction, to those of a recent study in which the effects of direct and indirect DA agonists on gnawing in C57Bl/6J mice were compared (Tirelli and Witkin, 1995). In this case, indirect agonists, but not direct agonists, induced gnawing and, furthermore, this behavior could not be elicited by combinations of D_1 and D_2 agonists. In squirrel monkeys, the indirect agonists cocaine and GBR 12909 have effects on directly observable behaviors that resemble those of D_1, but not D_2, agonists (Rosenzweig-Lipson *et al.*, 1994). Conversely, indirect agonists and D_2 agonists produce characteristic increases in FI-responding in squirrel monkeys, whereas D_1 agonists produce only decreases (Bergman *et al.*, 1995; Katz *et al.*, 1995). Although differential effects of indirect- and direct-acting agonists were found in the aforementioned studies, it should be emphasized that increases in eye blinks are produced by both D_1 and D_2 agonists, as well as nonselective DA agonists such as (−)-apomorphine.

Drug discrimination studies in primates also indicate that direct and indirect DA agonists have differential effects. For example, the direct DA agonists SKF 81297, SKF 82958, quinpirole, naxagolide and (−)-apomorphine and the indirect agonist methylphenidate substitute either completely or at least partially for the discriminative stimulus effects of cocaine (Kleven *et al.*, 1990; Spealman *et al.*, 1991) or the DA reuptake blocker, GBR 12909 (Melia and Spealman, 1991). In contrast, neither cocaine nor *d*-amphetamine substitute in monkeys trained to discriminate (−)-apomorphine (Woolverton *et al.*, 1987; Tang and Code, 1989). Additionally, cocaine, GBR12909 and *d*-amphetamine do not substitute in monkeys trained to discriminate SKF 81297 (Rosenzweig-Lipson and Bergman, 1993). The existence of this asymmetry in discriminative stimulus effects makes it clear that behavioral effects elicited by direct DA agonists cannot be entirely mimicked by indirect agonists, and offers another precedent for differential effects of direct and indirect agonists on spontaneous eye blinks.

The differential effects of direct and indirect agonists indicate either that indirect agonists do not act at sites involved in spontaneous eye blinks or that they produce other effects which prevent the expression of DA-mediated blinking. Because indirect agonists also bind to other monoamine reuptake sites with high affinity (Taylor and Ho, 1978), it is conceivable that indirect enhancement of noradrenergic or serotoninergic neurotransmission may mask DA-induced increases in eye blinking. However, the finding that the relatively DA-selective reuptake blocker GBR 12935 was not effective suggests that 5-HT does not play a role in limiting eye blink increases. In this study, the possibility that a common effect of the indirect agonists masks their ability to increase eye blinking in the monkey was examined by pretreating monkeys with cocaine before the administration of a behaviorally active dose of SKF 81297. Although a small reduction was observed after pretreatment with the highest dose of cocaine (2.5 mg/kg), overall, cocaine did not alter the blink-increasing effects produced by SKF 81297. Nor was there an *augmentation* of blink rate, as has been reported after combined treatment with SKF 81297 and quinpirole in Cebus monkeys withdrawn from haloperidol (Peacock *et al.*, 1990). Therefore, our results suggest that it is unlikely that another effect of indirect agonists prevents blink-rate increases.

An explanation for the findings that indirect agonists neither increase blink rate nor inhibit D_1 agonist-induced blinking is that DA reuptake sites are absent at the site(s) mediating DA-induced increases in spontaneous eye blinks. One possible site is the superior colliculus, a region that 1) is strongly implicated in spontaneous blinking (Karson, 1989), 2) contains low, but distinctly measurable populations of D_1 and D_2 receptors (Chivers *et al.*, 1984; Weller *et al.*, 1987; Camps *et al.*, 1989; Cortes *et al.*, 1989; Yokoyama *et al.*, 1994) and 3) has not been demonstrated to have DA transporters (Weller *et al.*, 1987). Evidence for the involvement of the superior colliculus in blinking is based on a decreased spontaneous blink rate in patients with progressive supranuclear palsy or tumors that affect the superior colliculus and a study in nonhuman primates showing that ablation of the superior colliculus causes long, blinkless staring spells (see Karson, 1989). Although it has been cited as an example of an area with mismatch between receptors and innervation (Yokoyama *et al.*, 1994), there is anatomical evidence for a DA-containing substantia nigra-superior colliculus pathway (Takada *et al.*, 1988; Moriizumi *et al.*, 1992). [With respect to the reduced blink rate in Parkinson's disease (Karson *et al.*, 1984) and in animal models (Colpaert *et al.*, 1991; Lawrence and Redmond, 1991), this pathway is destroyed by MPTP (Takada *et al.*, 1988)]. However, DA concentrations in the superior colliculus are extremely low (Weller *et al.*, 1987) and, possibly as a result of sparse innervation, transporter-mediated uptake of [^{3}H]dopamine cannot be demonstrated (Weller *et al.*, 1987). The apparent absence of reuptake sites in a highly implicated region such as the superior colliculus could explain our findings, yet it remains to be determined whether differential effects might be observed after the application of indirect- and direct-acting DA agonists in this site or in other regions implicated in spontaneous eye blinking (Karson, 1983).

In summary, this study reveals differential effects of direct and indirect DA agonists on spontaneous eye blinks in cynomolgus monkeys. The pharmacological characterization of DA-induced increases in blinking demonstrates that both selective and nonselective D_1 and D_2 agonists are effective and that low-efficacy agonists generally produced effects of a lower magnitude than those observed after (−)-apomorphine or quinpirole; in contrast, none of the indirect agonists produced increases in eye blinks. Differences between direct and indirect agonists could be expected based on previous studies comparing their discriminative stimulus and observable behavioral effects and the pharmacological mechanisms by which these compounds exert their actions. The findings of this study extend such differences to a behavior that is easily quantifiable in humans and in nonhuman primates.

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Send reprint requests to: Mark S. Kleven, Ph.D., Centre de Recherche Pierre Fabre, 17 avenue Jean Moulin, 81106 Castres Cedex France.