New Method for Assaying Antiapomorphine Activity in Pigeons

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Abstract □ An electronic monitoring system, which was capable of estimating and recording the intensity of the apomorphine-induced pecking syndrome, was evaluated with regard to its ability to function as an attendant-free assay instrument. Instrument responses were compared with responses derived from a visual recording technique which has been routinely used since 1960. The new monitoring system provides accurate and highly reliable estimates of the desired biological parameters.

Keyphrases □ Apomorphine-induced pecking syndromes, pigeons—assay method □ Pecking syndrome, pigeons—chlorpromazine HCl, methdilazine HCl inhibition □ Monitoring system, electronic—pecking syndrome, pigeons □ Inhibitor potency assay—apomorphine-induced pecking syndrome

Without doubt, the most familiar pharmacological property of apomorphine is its ability to evoke emesis in a variety of animal species. From pharmacodynamic, therapeutic, and toxicological standpoints, the emetic characteristics of the compound have been the subject of greatest attention through the years. On the other hand, interest in the nonemetic activities of apomorphine, particularly in animals refractory to its emetic propensities, has been sporadic, desultory, and largely uninspired. Some of these nonemetic responses are enumerated by Krueger *et al.* in their monumental "The Pharmacology of the Opium Alkaloids" (1).

Among the nonemetic responses to apomorphine is a curious "Zwangspicken" or compulsive pecking in pigeons, which was described by Amsler in 1923 (2). Interest in Amsler's "Zwangspicken" response in pigeons (for whom sublethal doses of apomorphine are *not* notably emetic) remained virtually nonexistent until 1957 when Burkman *et al.* (3) and Koster (4) described, in somewhat greater detail, this apomorphine-induced effect. The response was referred to by Koster as a "feeding hallucination" and by Burkman *et al.*, less imaginatively, as a "pecking syndrome."

Quantitative data describing the characteristics of the syndrome were published, simultaneously and independently, by Burkman (5) and by Dhawan and Saxena (6). Since then the syndrome has attracted increased attention as an example of drug-induced stereotypical behavior. The pecking syndrome is best characterized by a single symptom: a continuous, forceful, repetitive pecking by the pigeon on the floor, walls, and roof of the cage in which it is placed. Although the syndrome has been quantified in terms of intensity (7) and in terms of an all-or-none response (8), the intensity technique is regarded as both more reliable and more efficient than the quantal method. Therefore, the authors have directed most of their efforts toward improving their ability to monitor response magnitude.

The authors have successfully automated this method of quantifying the pecking syndrome and thereby have made what they believe to be a major technical improvement. Details relating to the construction of the instrument have been published (9).

This article describes and evaluates the automated instrument method for assessing the potency of apomorphine-inhibiting phenothiazines.

EXPERIMENTAL

Animals—Adult birds (1–2 years of age) of both sexes were housed in individual cages at a room temperature of 20–25° and conditioned to a 12-hr. light cycle. Birds retained for assay were those whose responsiveness to apomorphine HCl had been previously evaluated. Those that did not respond to intramuscularly administered doses of 0.5 mg./kg. were rejected as unsuitable. Thus, birds used in these experiments were *not* randomly selected from a general population, but rather were representative of a stock colony of birds having a high sensitivity to apomorphine.

All drugs used in the assays were dissolved in sterile 0.9% saline and injected intramuscularly.

Determination of Syndrome Intensity: Visual Technique—Each pigeon received the requisite dose of apomorphine HCl and was immediately placed in a wire mesh observation cage 22.9 × 38.1 × 22.9 cm. (9 × 15 × 9 in.). At 5–10-min. intervals, an observer made 1-min. counts of the bird's responses. The duration of observation was determined by the length of the period during which the animal exhibited the pecking syndrome. Since the syndrome was easily interrupted by sudden noises that distracted the bird, it was necessary to conduct the experiments in a sound-attenuated environment. The number of animals

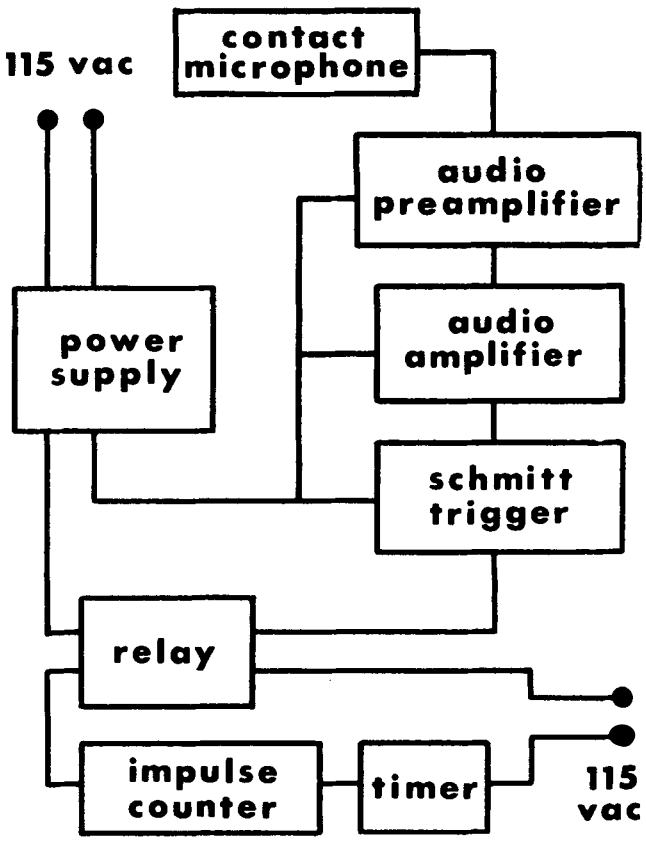

Figure 1—*Block diagram of one channel of the pecking syndrome monitor.*

Table I—Intensity of Apomorphine-Induced Pecking Syndrome as Determined by Two Monitoring Methods

	Method	
	Visual	Instrument
Number of pigeons	32	32
Mean cumulative response[a]	4395 ± 395	4440 ± 407
Coefficient of variation[b]	0.509	0.519

[a] Mean CPR ± standard error. Responses induced by apomorphine HCl, 0.5 mg./kg., i.m. No significant difference could be detected between methods. [b] Ratio of the standard deviation of a CPR sample to the mean CPR of that sample. No significant difference could be detected between methods.

that could be monitored simultaneously depended upon the observer's experience and his ability to make minute counts reliably. Generally, no more than six birds could be effectively monitored by a single person.

Response rate (pecks per minute) plotted against time from injection yielded a curve, whose parameters, suitably computed, provided such information as onset time, duration, peak rate, time-dependent rate changes, and the cumulative pecking response (CPR). The CPR, which has proved to be the most useful expression of response intensity, was determined by measuring the area under the rate–time curve with the aid of a compensating polar planimeter. Using a suitably calibrated instrument, the resulting total area was translated into total pecks, *i.e.*, the CPR.

Determination of Syndrome Intensity: Automated Instrument Technique—The design of the electromechanical CPR monitor was based upon the results of preliminary efforts to adapt an audio-pickup system for continuously recording the sharp, microphonically distinct clicks of the bird's beak as it struck the metal cage (9). The sounds that accompanied the compulsive pecking behavior provided a quantifiable variable which readily lent itself to machine processing.

To monitor apomorphine-induced pecking effectively, each channel of the system required: (*a*) a sensitive detector to pick up and transmit signals generated by sounds or vibrations developed as a result of the bird's pecking movements, and (*b*) a means for amplifying the detector signal so that (*c*) an electromechanical relay and impulse counter could be activated by each discrete pulse. Also essential was the incorporation of a device to improve the instrument's capacity to discriminate between pecking activity and fortuitous noises and movements unrelated to pecking.

The monitor design adopted for use is diagrammed in Fig. 1. The use of a simple contact microphone, bolted to the rear panel of a stainless steel observation cage 22.9 × 38.1 × 22.9 cm. (9 × 15 × 9 in.), served as a transducer. Environmental noises, bird vocalizations, and other air-transmitted sounds were incapable of activating the microphone. The unit, however, was sufficiently sensitive to pick up virtually all desired responses. Signals generated by the microphone were transmitted to a solid-state audiopreamplifier and audioamplifier. A Schmitt trigger, incorporated in the system, is a modified bistable multivibrator, which is widely used as a voltage discriminator in such devices as nuclear radiation pulse height analyzers. It served much the same function here. Input signals above a preset level provided an output signal sufficient always to activate the relay which, in turn, operated the impulse counter. This ensured that clean, artifact-free counts could be obtained. A 12- and 30-v. d.c. regulated power supply provided the energy for operation.

Although the monitor was incapable of being influenced by air-transmitted sounds, loud noises and strong visual stimuli easily distracted the animals under observation. This distraction was reflected in a distortion of the pecking response parameters. Thus, it was still necessary to control the environmental conditions under which responses were monitored. This was most easily accomplished by placing the caged pigeons in a sound-attenuating chamber constructed for that purpose. In addition, the monitor itself was placed in an adjoining room where it could be conveniently examined without disturbing the birds. This precaution further decreased the opportunity for the intrusion of environmental distortions.

Paired-Comparison Experiment—Evaluation of the utility of the pecking syndrome monitor was based on results of two experiments designed to determine the accuracy and reliability of the data supplied by the instrument.

The first experiment involved estimating syndrome intensity for a group of pigeons by recording their responses, using both the visual and instrument methods simultaneously. Thirty-two birds of mixed sex were injected intramuscularly with apomorphine HCl, 0.5 mg./kg., and placed in observation cages. The cages were placed, in turn, in the sound-attenuating chamber, and the monitors were activated. Observation ports allowed the experimenter to see the birds and to make the necessary visual counts. The CPR over its entire duration (about 2 hr.) served as the index of syndrome intensity, and this was determined for each bird by both methods. The conditions of the experiment satisfied the requirements of a "paired-comparison" design; evaluation of the visual and instrument methods was made by examining the magnitude of observed within-animal (between-methods) differences.

Inhibitor Potency Assay—The second experiment was a model assay similar to those repeatedly employed in studies of psycholeptic phenothiazines (10, 11). The apomorphine-inhibiting activities of two compounds, chlorpromazine HCl (CPZ) and methdilazine HCl (MDZ), were determined in terms of their ability to suppress the syndrome evoked by a standard dose of apomorphine HCl. The experiment was designed as a 4-point relative potency assay, utilizing 24 pigeons. Four groups of six birds received, initially, intramuscular injections of 0.5 mg./kg. of apomorphine HCl (1.6 μmole of base/kg.) 8 days prior to the start of the assay. The CPR values obtained served as controls against which the inhibiting influence of the phenothiazines was measured. On the assay day, each group of animals was premedicated with a single high or low dose of either CPZ or MDZ. All doses were expressed in terms of moles of base per kilogram body weight. Fifteen minutes after phenothiazine administration, apomorphine HCl, 0.5 mg./kg., was injected and the CPR was redetermined. Inhibition at each dose level was recorded as percent reduction of the control CPR. For each inhibitor, a log dose–probit response line was constructed, and the relative potency of MDZ was assessed using CPZ as the reference standard.

All activity data were collected by simultaneous use of visual and automated instrument techniques so that comparison could be made between methods.

RESULTS

Paired-Comparison Experiment—The characteristics of syndrome intensity are summarized in Table I. Thirty-two birds supplied two samples of CPR values; a comparison of these samples, in terms of sample means and dispersions about sample means, revealed that no significant difference existed. The computed t statistic had an associated $p > 0.6$. The visual and instrument techniques, therefore, provide equivalent estimates of syndrome intensity.

Inhibitor Potency Assay—The results are summarized in Table II. Both methods provided virtually identical estimates of median inhibitory doses (ID_{50}'s), slopes, and relative potency. The parameter estimates were so strikingly similar that a reliable conclusion could be drawn without recourse to further statistical manipulation and testing. No significant differences could be detected between the activity parameters derived from visual and instrument methods.

DISCUSSION

Although the visual technique for quantifying pecking syndrome intensity employed in earlier studies has proved to be highly reliable, its use requires an experienced observer, one who has been trained to make selective, unbiased, and reproducible counts. Even under the best conditions, an observer can usually program the simultaneous visual monitoring of no more than six birds. Under standardized conditions of the phenothiazine assays, the period of observation lasts approximately 2 hr., and the preliminary animal weighings and premedication increase the period to about 2.5 hr. Thus, the number of man-hours required to satisfy the needs of even a comparatively simple 4- or 6-point crossover relative potency assay becomes enormous, and the assay technique has been relatively inefficient in terms of the utilization of an investigator's time.

This kind of inefficiency now seems unnecessary, since the syndrome can be readily quantified by an instrument method which does not require the full and constant attention of the investigator during the recording session. Furthermore, use of the instrument that automatically accumulates and records compulsive pecking

Table II—Suppression of Apomorphine-Induced Pecking Syndrome by Chlorpromazine (CPZ) and Methdilazine (MDZ) Assayed by Two Methods

Statistic	Method	
	Visual	Instrument
ID_{50} (CPZ)[a]	2.05 (1.10–3.80)	1.65 (0.90–2.95)
ID_{50} (MDZ)	29.8 (24.0–37.0)	27.1 (20.9–34.6)
Slope (CPZ)[b]	84.9 ± 67.5	92.9 ± 29.1
Slope (MDZ)	71.6 ± 47.4	82.6 ± 30.7
Common slope (CPZ) + (MDZ)	90.11	78.43
Relative potency[c]	0.072 (0.046–0.100)	0.063 (0.043–0.96)

[a] Median inhibiting dose (95% confidence limits); in unit of micromoles of base per kilogram body weight. [b] Slope of the dose–response line ± 95% confidence limits; in units of percent/log dose. [c] Potency of MDZ (95% confidence limits) relative to CPZ.

activity obviates deriving CPR values by curve plotting and area computing manipulations. And, finally, the comparatively unsophisticated device can be fabricated with standard and readily available components.

It seems clear from the results summarized in Tables I and II that the instrument monitor provides data as accurate and as reliable as those provided by the more tedious visual method. The results of the inhibitor potency assay, particularly, demonstrate the utility and applicability of the instrument method for such experiments.

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 3, 1970, from the *Division of Pharmacology, College of Pharmacy, Ohio State University, Columbus, OH 43210*

Accepted for publication June 17, 1970.

The authors are indebted to Dr. W. R. Williams, Mead Johnson Research Center, Evansville, Ind., for the supply of methdilazine hydrochloride (Tacaryl).

This investigation was supported by Grant MH 11733 from the U. S. Public Health Service.

Drug Permeation through Thin Model Membranes I: Development of a Polymeric Model Biomembrane

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Abstract □ The development of a polymeric nonporous model membrane, containing natural membrane components, and its use in a two-compartment transport cell are reported. Consideration was given to the polymer used to form the polymer matrix, membrane thickness, the amount and type of biological material incorporated, and the effect of nonbiological additives. The effect of these changes on the transport properties of the various membranes were monitored in terms of k_d, the rate of disappearance constant of salicylic acid, from the pH 2.0 compartment of the transport cell. As a result of these studies, a standard model biomembrane was designed, containing 44% ethylcellulose, 44% biological materials, and 12% mineral oil, dry weight of the membrane. From the lack of solvent flux under experimental conditions and the first-order disappearance of salicylic acid, it appears that the polymer membrane mimics the functionality of natural membranes insofar as passive diffusion is concerned.

Keyphrases □ Biomembrane model, polymeric—drug permeation □ Membrane, nonporous—natural membrane components □ Drug absorption, passive—model membranes □ Transport rates—drugs through model membranes

Model membranes have been employed in attempts to develop *in vitro* model systems whose transport characteristics correlate with *in vivo* passive drug absorption. In addition to permitting systematic study of the many variables affecting the *in vivo* process, these model systems also act as a potential tool for assessing the ability of new medicinal agents to cross the gastrointestinal membranes and similar barriers. Although considerable work has been reported concerning a number of models, significant accomplishments are yet to be made in elucidating the transport process occurring *in vivo* and in producing *in vitro* models that more closely reflect the functionality of biological membranes.

The following properties may be considered desirable in any model system for passive drug absorption:

1. The membrane should be thin, with a low volume ratio of membrane to surrounding aqueous media. This elimination of the membrane "volume" significantly reduces drug retention within the membrane phase.

2. Transport selectivity of the drug(s) in question should be based on solubility in a homogeneous barrier rather than on dialysis through a microporous structure.

3. The membrane should be sufficiently durable to withstand extended experimental procedures without loss of integrity which might lead to changes in the transport characteristics of the membrane.

4. It should be possible to demonstrate a correlation between *in vitro* transport rates and *in vivo* absorption rates.

Model membranes can be conveniently classified into two groups. First, some membranes are essentially bio-