Apomorphine: Modification of Its Hyperthermic Effect in Rabbits by p-Chlorophenylalanine

Abstract. *The hyperthermic response of rabbits to apomorphine, a dopaminergic agonist, is abolished by prior treatment with* p-*chlorophenylalanine. If such 5-hydroxytryptamine (5-HT)-depleted animals are administered a peripherally acting decarboxylase inhibitor plus 5-hydroxytryptophan, central stores of 5-HT are regenerated and the hyperthermic response to apomorphine is restored in part. The effects of apomorphine in rabbits with elevated concentrations of 5-HT are not different from those in control animals. The behavioral effects of apomorphine appear to be constant in all groups of animals tested. It is suggested that the hyperthermic effects of apomorphine in rabbits require the presence of 5-HT.*

Central dopaminergic systems have been implicated in the actions of apomorphine in a number of species. Various drug effects, such as changes in body temperature, hypermotility, compulsive gnawing, and other stereotypic behavior, have been established as being dopaminergic in nature (*1*). Supporting evidence for such a concept emerged after the development of butyrophenones and diphenylbutylamines, compounds which possess rather effective and selective dopaminergic receptor blocking properties (*2*).

Dopamine is one of several monoamines found in the brainstem that have been assigned a thermoregulatory role in rabbits. Dopamine and 5-hydroxytryptamine (5-HT) have been postulated to be involved in increases in body temperature while norepinephrine is implicated in the mediation of mechanisms involved in the lowering of body temperature (*3*).

Apomorphine and *d*-amphetamine are two agents capable of exerting a hyperthermic effect in rabbits. It is evident that the temperature responses result from activation of central dopaminergic mechanisms since the prior administration of pimozide, a selective dopaminergic receptor blocking agent, abolishes the apomorphine- and *d*-amphetamine-induced hyperthermias (*4*). However, since dopamine is only one of two hyperthermic neuroamines in the rabbit central nervous system, we decided to investigate whether 5-HT might also be involved in the hyperthermic response to apomorphine.

p-Chlorophenylalanine (*p*CPA) is a specific inhibitor of tryptophan hydroxylase, the enzyme governing the rate-limiting step in the 5-HT biosynthetic pathway (*5*). If this monoamine were involved in the apomorphine-induced hyperthermic response, then rabbits that have received *p*CPA should respond to the drug in a modified manner.

Male New Zealand rabbits (Totem Farms, Washington), weighing between 2.0 and 2.3 kg, were used in these studies. All experiments were conducted in a temperature-controlled environment of 22.0° ± 1.0°C. Animals were restrained in open wooden stanchions, to which they had previously been conditioned, and were also supplied drinking water without restriction. Changes in rectal temperature were monitored by a telethermometer (Yellow Springs Instruments) and automatic recorder (Leeds and Northrup). The following drugs were dissolved in 0.9 percent saline solution: apomorphine hydrochloride (Merck Sharp & Dohme); *p*-chlorophenylalanine methyl ester hydrochloride (Nutritional Biochemicals); L-α-methyl-α-hydrazino-β-(3,4-dihydroxyphenyl)propionic acid (more commonly designated as L-α-hydrazino-α-methyl-dopa, or HMD) (gift from Merck Sharp & Dohme Research Laboratories); and 5-hydroxy-DL-tryptophan (5-HTP) (Regis).

In our procedure we observed and compared the effects of apomorphine in (i) control rabbits, (ii) those depleted of 5-HT, (iii) those in which 5-HT had been restored, and (iv) those in which 5-HT was elevated. Concentrations of 5-HT in brainstems of animals in each of the four groups were determined by a modification of the method of Bogdanski *et al.* (*6*). Care was taken to remove excessive 5-HTP by three washings of the butanol fraction with borate buffer.

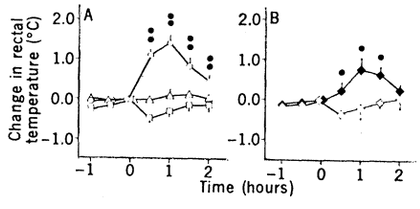

Fig. 1. (A) Changes in rectal temperature in response to apomorphine (4.0 mg/kg, intravenously) in controls (open circles) ($N = 10$) and rabbits pretreated with *p*CPA (open squares) ($N = 8$). Also shown is the response to saline in rabbits pretreated with *p*CPA (open triangles) ($N = 3$). (B) Changes in rectal temperature in response to apomorphine (4.0 mg/kg, intravenously) in rabbits pretreated with *p*CPA after HMD and 5-HTP (closed diamonds) ($N = 4$) and in rabbits pretreated with *p*CPA after saline (open diamonds) ($N = 4$). Vertical lines indicate standard error of the mean. Significance of difference: (A) ● ●, $P < .01$; (B) ●, $P < .05$.

An intravenous injection of apomorphine, 4.0 mg per kilogram of body weight (base weight), in the rabbit elicits pupillary dilatation, compulsive gnawing, and an elevation in rectal temperature. The vascular changes as well as the apomorphine-induced gnawing, hyperactivity, and hyperreactivity all subside approximately 60 minutes after the injection. The temperature response consists of a hyperthermia of approximately + 1.5°C in magnitude, the peak occurring at about 45 to 60 minutes and lasting slightly over 2 hours in duration.

Rabbits pretreated with *p*CPA methyl ester (200 mg/kg, administered intraperitoneally at 48, 24, and 12 hours) exhibit no hyperthermia in response to apomorphine but rather respond with a slight, though statistically significant, reduction in rectal temperature (Fig. 1A). The behavioral and stereotypic effects of apomorphine appear to remain intact in these rabbits depleted of 5-HT.

Animals that received prior treatment with *p*CPA in the above experiment were randomly divided into two groups of four animals each. The first group was administered HMD (25 mg/kg, intraperitoneally) followed 60 minutes later by 5-HTP (10 mg/kg, intravenously). After three additional hours to allow for adequate regeneration of central 5-HT stores, a second injection of apomorphine was administered. The second group of animals received injections of saline solution in lieu of HMD and 5-HTP. Three hours later, they too were challenged with a second administration of apomorphine. In rabbits in which 5-HT was restored, a second injection of apomorphine produced an increase in rectal temperature. Animals receiving saline, and hence remaining depleted of 5-HT, continued to respond with a drop in temperature (Fig. 1B). Animals in each group exhibited similar behavioral signs in response to apomorphine.

Elevated concentrations of 5-HT were obtained in animals by means of

pretreatment with HMD 1 hour prior to administration of 5-HTP. When challenged with apomorphine 3 hours later, these rabbits displayed hyperthermic and behavioral responses indistinguishable from the responses of rabbits that had not been pretreated.

Table 1 shows the comparison between concentrations of 5-HT in the brainstems of animals from all four groups. Concentrations of 5-HT are reduced to approximately 30 percent of normal after pretreatment with *p*CPA at the doses and times described above. Administration of HMD and 5-HTP to animals pretreated with *p*CPA restored concentrations of 5-HT to their normal levels. Elevation of 5-HT by means of HMD and 5-HTP alone produced a 60 percent increase over control levels.

Our results indicate that 5-HT has an essential role in the mediation of the hyperthermic response of rabbits to apomorphine (*7*). This conclusion is based on the finding that, after depletion of cerebral 5-HT stores by *p*CPA, apomorphine is unable to elicit its characteristic increase in rectal temperature. However, after central 5-HT concentrations are restored to normal, apomorphine can again induce a hyperthermia. Although this restored response is dissimilar to the control response in magnitude and duration, the intimate involvement of 5-HT in the hyperthermic response to apomorphine is clearly implicated. The differences between control and restored responses may reflect insufficient replenishment of 5-HT stores in specific brain regions. The uptake of 5-HTP into adrenergic and dopaminergic nerve terminals and subsequent conversion to 5-HT via the action of ubiquitous *l*-aromatic amino acid decarboxylase may contribute to the increase of total 5-HT in the brainstem.

A number of laboratories have reported the failure of 5-HT depletion with *p*CPA to disrupt stereotypic and gnawing behaviors induced by apomorphine in rats (*8*). This is, however, inconsistent with a recent report in which midbrain raphe lesions selectively blocked apomorphine-induced gnawing in rats (*9*). In our study, the behavioral effects of apomorphine in rabbits appear to be independent of central 5-HT, inasmuch as the behavior of animals after injection in all four groups was essentially identical.

Our hypothesis that apomorphine exerts its hyperthermic action via a 5-HT mechanism is consistent with other studies from our laboratory which indicate tryptaminergic neuronal substrates for certain agents that alter thermoregulation (*10*). This is, however, to our knowledge, the first finding of a dopaminergic–5-HT interaction in temperature responses to pharmacologically active agents (*11*).

Table 1. Changes in concentrations of brainstem 5-HT after various pretreatments. Values are expressed as the means ± the standard error of the mean (S.E.M.); *N* indicates the number of animals used.

Prior treatment	*N*	5-HT content (μg/g) ± S.E.M.	Control level (%)
None	9	0.58 ± 0.03	100
*p*CPA	11	.18 ± .03*	31
*p*CPA + HMD + 5-HTP	7	.61 ± .04	105
HMD + 5-HTP	6	.95 ± .06*	164

* $P < .01$, compared to no pretreatment.

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11. While this report was in press, M. Grabowska, J. Michaluk, and L. Antkiewicz [*Eur. J. Pharmacol.* **23**, 82 1973)] suggested that the apomorphine-induced hypothermia in rats and mice is likewise dependent upon brain 5-HT. This conclusion was based on their findings of (i) increased 5-HT turnover concomitant with the hypothermia, (ii) blockade of both increased 5-HT turnover and hypothermia by the dopaminergic receptor blockers spiroperidol and pimozide; and (iii) blockade of both increased 5-HT turnover and hypothermia by the 5-HT receptor blocker LSD.
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Cerebroside Antibody Inhibits Sulfatide Synthesis and Myelination and Demyelinates in Cord Tissue Cultures

Abstract. *Antiserum to cerebroside was prepared in rabbits by injection of cerebroside together with bovine serum albumin in complete Freund's adjuvant. When applied to cultures of embryo mouse spinal cord at explantation, this antiserum inhibited sulfatide synthesis and myelination; when applied to myelinated cultures it inhibited sulfatide synthesis and produced demyelination. Complement fixation assays also show antibody to cerebroside in serums from rabbits with experimental allergic encephalomyelitis induced by injection of whole white matter. Absorption of such serum with cerebroside abolishes the inhibiting and demyelinating activities.*

Myelinated cultures of mammalian central nervous system (CNS) tissue become demyelinated when exposed to serum from rabbits with experimental allergic encephalomyelitis (EAE serum) induced by injection of whole white matter in complete Freund's adjuvant (CFA) (*1*). Others have shown that such serums contain antibodies of at least two different specificities, namely, antibodies against the encephalitogenic basic protein and cerebroside (*2*, *3*). Seil *et al.* (*4*) have reported that serums from guinea pigs sensitized with myelin basic protein did not demyelinate CNS tissue cultures. However, Yonezawa *et al.* (*5*) reported that demyelination did occur when serums from guinea pigs or rabbits sensitized with myelin basic protein was added to CNS cultures. Bornstein (*6*) has also found that serums from rabbits inoculated with basic protein in CFA are capable of demyelinating cultured mouse spinal cord but that this effect is demonstrated less consistently than with serums from rabbits exposed to whole tissue in CFA. In 1970, Dubois-Dalcq *et al.* (*7*) showed that antiserums to cerebroside produced demyelination in CNS tissue cultures.

Bornstein and Raine (*8*) showed that