Biochemical Pharmacology, Vol. 20, pp. 1889–1900. Pergamon Press, 1971. Printed in Great Britain

EFFECT OF TRANQUILIZERS AND ANTIDEPRESSANTS ON GLYCOGEN PHOSPHORYLASE OF RAT BRAIN

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(*Received* 5 *June* 1970; *accepted* 20 *November* 1970)

Abstract—Tranquilizers and antidepressants were investigated for their effect on the glycogen phosphorylase (α-1,4-glucan: orthophosphate glucosyltransferase; EC 2.4.1.1) activity of rat brain after sacrificing the animal with liquid nitrogen. The tranquilizers consisted of reserpine and four phenothiazines. Isoreserpine and promethazine, both not considered tranquilizers, were included as controls. All tranquilizers except prochlorperazine depressed the phosphorylase activity. The controls did not affect the phosphorylase level. A tentative explanation for the lack of effect of prochlorperazine is given. The antidepressants consisted of: two monoamine oxidase inhibitors, pargyline and iproniazid; a tricyclic antidepressant, desmethylimipramine; and a psychic stimulant, amphetamine. The antidepressants acted counter to tranquilizers, i.e. they enhanced the absolute or relative level of phosphorylase activity. However, the enhancing effect was best observed when rats were treated with reserpine before or after the drug. A unified explanation for the effect of most of both tranquilizers and antidepressants on the phosphorylase activity is given, based on a reasonable mode of action and a postulate stating that the phosphorylase activity is correlated with the availability of norepinephrine at the adrenergic receptor sites. These facts suggest that the phosphorylase activity of brain may be a barometer for CNS adrenergic activity. The results suggest that the glycogen phosphorylase of brain may be involved in actions of many drugs used as tranquilizers and antidepressants.

WE HAVE had certain reasons, albeit speculative, to entertain the view that glycogen phosphorylase (α-1,4-glucan: orthophosphate glucosyltransferase; EC 2.4.1.1) might be involved in mental illness. A feasible approach to test this view is to study the effect on the brain phosphorylase of animals of centrally acting drugs that relieve symptoms of mental illness or elicit symptoms reminiscent of those observed in mental illness. In previous reports, we have shown that the phosphorylase level of rat brain is depressed by psychotomimetic drugs and enhanced by phenothiazine type of drugs,[1,2] tending to support the correctness of our avenue of approach.

Since the publication of the above papers, it was found that the particular method used in sacrificing rats affected not only the magnitude but also the direction of the effect of drugs on the phosphorylase level. In the former method (herein called the chloroform method), chloroform was used for sacrificing the rats, whereas in the newer method (herein called the liquid nitrogen method), rats were sacrificed by immersion in liquid nitrogen. The latter method is believed to freeze the enzymic state as it existed prior to sacrifice and, therefore, the observed values may reflect more truly the state *in vivo*. Our most recent paper[3] serves as a bridge between the investigations using the chloroform method and the liquid nitrogen method. In the above paper, we showed that chlorpromazine enhanced the phosphorylase level by the

chloroform method and depressed it by the liquid nitrogen method, and further, that chloroform *per se* is not involved in the effect on the enzyme. The observed reverse relationship of drug effect on the phosphorylase activity holds for all the phenothiazine drugs used in the present experiment (unpublished observation), but whether it holds for all other types of drugs cannot be said categorically.

Our results to date suggest the existence of a meaningful relationship between certain centrally acting drugs and their effect on the phosphorylase level of rat brain.

There are a few reports on the effect of centrally acting drugs on the brain phosphorylase, but these are diverse and conflicting. Breckenridge and Norman[4] reported that reserpine and chlorpromazine decreased the enzyme activity, but that amphetamine and a monoamine oxidase inhibitor failed to change the activity of this enzyme. In 1965, the same authors[5] reported enhancement of brain phosphorylase activity after amphetamine, but no change after reserpine. Belford and Feinleib[6] obtained equivocal results with reserpine, i.e. lowering of enzyme activity in the first series of experiments and no change in a new series; moreover, they reported no change after either iproniazid or chlorpromazine. Estler[7] reported enhancement of the phosphorylase activity in mice, given morphine in doses that increased the motor activity, and reduction of enzyme activity in rats sedated by morphine injection. The reason for this diverseness of the results is probably due in large measure to various methods used in obtaining the specimens for the phosphorylase assay, and possibly due also to dosages and timing of assay.

In the present paper, we have examined by the liquid nitrogen method the effect of drugs that may be classified as tranquilizers and as antidepressants. It will be shown that these two classes of drugs, which may be considered as having opposite clinical effect, also have an opposite effect on the phosphorylase activity of rat brain.

METHODS

Female albino rats of Wistar strain weighing between 160 and 220 g were used in all experiments. They were fasted 20–24 hr prior to sacrifice. The animals were sacrificed by immersion in liquid nitrogen for 3 min and were stored at −40° until brains were removed for the phosphorylase assay. The assays were completed usually within 3 or 4 days. Decline in enzyme activity during the storage was negligible. The brain was removed by using a small spatula-like instrument in a cold room after sawing the head off and cleaving the skull sagittally in half with a sharp knife with thin blade (made from a saw). The removed brain was kept frozen until homogenized. Usually two brains were homogenized for the first set of assays immediately after their removal from the skull and two brains 3 hr later.

Phosphorylase assay. The phosphorylase activity was measured by a modification of the method of Cori *et al.*,[8] the details of which were previously reported.[3] There was a further minor change in that only Norit A was used to remove nucleotides from all brain homogenates as well as from glycogen. Such removal is imperative if the differential effect of drugs on phosphorylase is to be detected, especially if the magnitude of the effect is small. The activity was expressed in phosphorylase units[9] per milliliter of the enzyme homogenate. A phosphorylase unit $= 1000 \times 1/t \log [Xe/(Xe-X)]$, where $t = 15$ min and $Xe =$ per cent conversion of glucose-1-phosphate at equilibrium. $X =$ per cent of the initial glucose 1-phosphate which had reacted after 15 min of incubation. The ratio, units of phosphorylase determined without the

addition of AMP to units of phosphorylase determined with AMP, times 100 is a measure of the relative amount of the active form *a* to the total phosphorylase in per cent. Comparison of the effect of treatment to a control was made by using the activity of phosphorylase *a*, i.e. the activity observed without the addition of AMP. This method of assessing drug effects stems from our previous experience with LSD-25. This drug affected the activity of phosphorylase *a* as well as that of the total enzyme.[2]

All solutions used in enzyme reactions were prepared in glass-distilled water.

Validation of the use of liquid nitrogen with respect to phosphorylase levels in vivo. Since the brain phosphorylase levels obtained when rats are sacrificed by immersion in liquid nitrogen are rather high, a question arises if they represent the true state of the enzyme *in vivo*. Since the effect of endogenous AMP on the phosphorylase level can be discounted, because it is routinely removed from all homogenates by the use of Norit A, the brain either has high levels intrinsically or they rise during the brief period of freezing. In our previous investigation,[3] presence or absence of respiration was found to have a profound effect on phosphorylase levels so that unless the brain freezes before the effect of cessation of respiration takes place the phosphorylase levels would rise, possibly to those levels observed in brains removed under anesthesia (80 mg/kg, i.p.) and then frozen (Table 1). These levels were found to be very high, probably reflecting the effect of cessation of respiration and of anoxia when the brains were severed from the skulls. If the level *in vivo* is low, it should be possible to

TABLE 1. EFFECT OF ACCELERATED FREEZING OF RAT BRAIN ON PHOSPHORYLASE ACTIVITY

Rats*	−AMP† (Mean ± S.E.M.) (phosphorylase units)	+AMP‡ (Mean ± S.E.M.) (phosphorylase units)	$\frac{-AMP}{+AMP} \times 100$ (Mean ± S.E.M.)
Saline (5)	104·8 ± 2·3	182·8 ± 5·7	57·46 ± 1·38
Pentobarbital (5)	109·8 ± 5·2	183·2 ± 4·8	59·88 ± 1·68
Pentobarbital + scalp removal (5)	101·8 ± 5·5	176·8 ± 5·5	57·48 ± 1·55
Brains removed and then frozen § (11)	146·6 ± 4·0	227·0 ± 3·1	64·57 ± 1·16
Brains frozen *in situ* and then removed ‖ (2)	96·0 ± 2·0 P < 0·001	194·0 ± 18·0	49·75 ± 3·55 P < 0·001

* Rats were divided into three groups (first three rows). The saline-injected group was sacrificed by immersion in liquid nitrogen. The pentobarbital group (35 mg/kg, i.p.) was sacrificed 30 min after injection by the same method. The third group of rats had their scalp removed under pentobarbital anesthesia (35 mg/kg, i.p.) exposing the thin skull which was coated with glycerine before they were sacrificed in liquid nitrogen. These procedures were expected to accelerate freezing time appreciably. After freezing, the brains were removed and the phosphorylase activity was assayed with and without the addition of AMP in reaction mixtures. No differences were observed among the three groups. Figures in parentheses give number of animals.

† Phosphorylase determination in the absence of AMP.

‡ Phosphorylase determination in the presence of AMP.

§ Brains were removed under pentobarbital anesthesia (80 mg/kg, i.p.) and then frozen in liquid nitrogen. Note the high phosphorylase levels compared to any other levels found in this table or in subsequent tables.

‖ Rats received pentobarbital (80 mg/kg, i.p.) and were sacrificed in liquid nitrogen 15 min after injection. Then brains were removed and assayed.

observe a lower level than that usually obtained with liquid nitrogen if the freezing process is accelerated. We have tested this assumption. Rats were divided into three groups. One group was sacrificed by immersion in liquid nitrogen. The second group received pentobarbital (35 mg/kg, i.p.) and was sacrificed 30 min after injection. The third group of rats had their scalp removed under pentobarbital anesthesia (35 mg/kg, i.p.), exposing the thin skull, which was coated with glycerine before sacrificing them. The removal of the scalp as well as the coating of the skull with glycerine would hasten the cooling time, for glycerine shortens the cooling time appreciably when the material to be frozen in liquid nitrogen is precoated with it.[10] As may be seen from Table 1, no significant differences were observed among the three groups, suggesting that the observed levels are equal to or not much higher than the levels *in vivo*. Incidentally, the brain phosphorylase of rats that were treated exactly like the ones which received 80 mg/kg, i.p., of pentobarbital but which were killed in liquid nitrogen before brains were removed had significantly lower activity (even lower than the usual normal levels) than that of the rats whose brains were removed and then frozen (Table 1).

Experimental design and statistics. In one experiment, a 6 × 6 Latin-square design was used and in another experiment a 4 × 8 randomized complete-block design was used.[11] Homogeneity of error variance for all treatment groups was assumed. This error variance was used in comparing the control with each of the treatment groups by the least significant difference method, which is basically a Student's *t*-test using a pooled error variance.[11] The standard error of the estimate of mean value (S.E.M.) was calculated using the pooled error variance by the formula, $s_{\bar{x}} = \sqrt{s^2/r}$, where $s_{\bar{x}}$ is error variance, and r is the number of replications in a treatment group.[11] Hence, all treatment groups have an identical S.E.M.

In other experiments, control and experimental animals were intermixed randomly as to sequence of assay, using the same batch of animals of approximately similar weight. Comparison of a control with a treatment group was carried out according to the Student's *t*-test. Such comparison is valid only within the experimental design for which the control was used, because the numerical values of phosphorylase activity are affected by many factors, such as the kind of instrument and chemicals used in the assay, weights and age of the animals, the person who carries out the assay, etc. In any one experimental design, the experimental conditions were kept as uniform as possible.

Drugs. The drugs used were: perphenazine (solution for injection prepared by dissolving first in absolute ethanol, diluting with water and acidifying with 0·1 N HCl till turbidity disappeared), triflupromazine, prochlorperazine ethanedisulfonate, promazine hydrochloride, promethazine hydrochloride, chlorpromazine hydrochloride, iproniazid phosphate, pargyline hydrochloride, *d*-amphetamine hydrochloride (Nutritional Biochemical Corp.); reserpine (Nutritional Biochemical Corp.; solution for injection made by first dissolving in glacial acetic acid and then diluting to required volume with water, e.g. 4 mg reserpine, 0·2 ml glacial acetic acid, 9·8 ml water); 3-isoreserpine (Ciba, Su-3313; solution for injection prepared exactly like that for reserpine); desmethylimipramine and sodium pentobarbital (Nembutal, Abbott). Where applicable, drug doses are expressed as salts.

Chemicals. Norit A (an acid-washed product, further washed with distilled water, and finally with glass-distilled water) was obtained from Pfanstiehl Laboratories.

Glycogen [from shell fish; nucleotides contained as impurities in glycogen removed at the time of preparing its 4% (w/v) solution by treating the solution with 1% (w/v) Norit A and centrifuging at 8200 *g* for 30 min] was obtained from Nutritional Biochemical Corp.

RESULTS

Effect of tranquilizers on phosphorylase activity

Reserpine. Reserpine depressed the brain phosphorylase activity whether given in a dose of 5 mg/kg, s.c., and the phosphorylase activity observed 24 hr later or in a dose of 5 mg/kg, i.p., and the activity assayed 3 hr after injection (Table 2). In later experiments, it was found that at 3 hr a dose of 3 mg/kg, i.p., was just as effective as 5 mg/kg, i.p. No other agent was as powerful as reserpine in its depressing effect on the phosphorylase activity, either in magnitude or duration.

TABLE 2. EFFECT OF RESERPINE AND ISORESERPINE ON PHOSPHORYLASE LEVEL OF RAT BRAIN

Rats*	Dose (mg/kg)	Time after injection (hr)	−AMP† (Mean ± S.E.M.) (phosphorylase units)	+AMP (Mean ± S.E.M.) (phosphorylase units)	$\frac{-AMP}{+AMP} \times 100$ (Mean ± S.E.M.)
Control (11)		24	115·5 ± 2·7	201·4 ± 2·7	57·43 ± 1·30
Reserpine (13)	5 (s.c.)		91·0 ± 5·3	191·7 ± 4·0	47·19 ± 2·15
			P < 0·01		P < 0·001
Control (8)			113·4 ± 4·0	192·4 ± 3·0	58·96 ± 1·88
Reserpine (8)	5 (i.p.)	3	87·1 ± 4·0	191·0 ± 3·0	45·53 ± 1·88
			P < 0·001		P < 0·001
Control (6)			113·5 ± 2·4	190·7 ± 3·9	59·67 ± 1·23
Reserpine (11)	3 (i.p.)	3	89·9 ± 1·8	192·4 ± 3·3	46·78 ± 0·76
			P < 0·001		P < 0·001
Control (10)			104·6 ± 3·9	180·9 ± 4·2	57·74 ± 1·45
Isoreserpine (12)	5 (i.p.)	3	108·3 ± 3·0	182·0 ± 4·4	59·62 ± 1·23

* Rats were injected with drugs and at designated times they were sacrificed by immersion in liquid nitrogen. Then brains were assayed for phosphorylase activity.

† For legends, see Table 1.

In contrast to reserpine, its stereoisomer, isoreserpine, has no sedative action.[12] As may be seen in Table 2, this drug had no effect on the phosphorylase activity.

Phenothiazines. We tested by a 6 × 6 Latin-square design the effect of five drugs and a saline control, listed in Table 3, on the brain phosphorylase activity. Dosages were uniformly 10 mg/kg, s.c., and the phosphorylase was assayed 4 hr after the administration of drugs. Promethazine, a drug not generally considered useful clinically in the treatment of psychosis[13–15] but known for its antihistaminic property, is not a tranquilizer, although it resembles chlorpromazine structurally in having an aliphatic side chain. Hence, this drug was not expected to show any effect on the phosphorylase activity. As may be seen from Table 3, promethazine did not depress the phosphorylase activity, whereas promazine and trifluprazine, the two aliphatic side chain derivatives similar to promethazine, depressed the phosphorylase activity significantly. A piperazine derivative, perphenazine, also depressed the phosphorylase

activity but not as much as the aliphatic derivatives. Prochlorperazine, another piperazine derivative, did not affect the phosphorylase activity.

Effect of antidepressants on phosphorylase activity

Monoamine oxidase inhibitors. Two monoamine oxidase inhibitors of different chemical structure, namely, iproniazid and pargyline, were tested. Both drugs were investigated in single and multiple doses. In the single dose experiments (herein called acute), the doses were 100 mg/kg, s.c., for both drugs, and the animals were sacrificed 4 hr after the administration of drugs. In the multiple dose experiments (herein called chronic), the dosage schedule for iproniazid was 100 mg/kg the first day and 50 mg/kg daily for the next 3 days, with the last dose given 4 hr prior to sacrifice. All doses were administered subcutaneously. The schedule for pargyline was 25 mg/kg, s.c., daily for 4 days, with the last dose given 4 hr prior to sacrifice. The results are shown in Table 4. Significant increases in phosphorylase levels were observed after chronic administration of both iproniazid and pargyline. Compared to iproniazid, pargyline appeared to be more potent. After a single dose, neither iproniazid nor pargyline affected the phosphorylase activity.

TABLE 3. EFFECT OF PHENOTHIAZINES ON PHOSPHORYLASE LEVEL OF RAT BRAIN*

Treatment	−AMP (Mean ± S.E.M.) (phosphorylase units)	+AMP (Mean ± S.E.M.) (phosphorylase units)	$\frac{-AMP}{+AMP} \times 100$ (Mean ± S.E.M.)
Control (6)	127·8 ± 4·5†	213·3 ± 5·8	60·00 ± 1·60
Promethazine (6)‡,§	119·3 ± 4·5	207·8 ± 5·8	57·48 ± 1·60
Prochlorperazine (6) ‖	119·5 ± 4·5	210·8 ± 5·8	56·65 ± 1·60
Perphenazine (6)‖	113·8 ± 4·5 P < 0·05	202·8 ± 5·8	55·90 ± 1·60
Promazine (6)‡	109·1 ± 4·5 P < 0·01	208·0 ± 5·8	52·52 ± 1·60 P < 0·01
Triflupromazine (6)‡	109·5 ± 4·5 P < 0·01	203·3 ± 5·8	53·98 ± 1·60 P < 0·02

* A 6 × 6 Latin-square design was used for the experiments. Doses for phenothiazine drugs were all 10 mg/kg, s.c., and the rats were killed 4 hr after drug administration by immersion in liquid nitrogen. Brain phosphorylase was assayed as explained in Table 1.

† See text for explanation of identical S.E.M. as well as for high control value.

‡ Aliphatic side chain derivative.

§ Preliminary independent assay showed also that this drug had no phosphorylase depressing effect (P > 0·4).

‖ Piperazine side chain derivative.

Interaction of amphetamine and pargyline with reserpine. A 4 × 8 randomized complete-block design was used. Amphetamine (10 mg/kg, i.p.), like iproniazid and pargyline after a single dose, failed to enhance the phosphorylase level when determined 20 min after injection, at which time the effect of the drug on behavior was at its maximum (Table 5). However, when reserpine was administered to the rat prior to amphetamine or after pargyline, the brain phosphorylase level was affected in a manner that suggested a relationship between the change in level and the behavior.

TABLE 4. EFFECT OF MONOAMINE OXIDASE INHIBITORS ON PHOSPHORYLASE LEVEL OF RAT BRAIN

Treatment	−AMP (Mean ± S.E.M.) (phosphorylase units)	+AMP (Mean ± S.E.M.) (phosphorylase units)	$\frac{-AMP}{+AMP} \times 100$ (Mean ± S.E.M.)
Control (8)	110·9 ± 3·8	187·0 ± 4·8	59·16 ± 1·69
Iproniazid (8)* (acute)	110·9 ± 2·9	184·8 ± 2·5	60·09 ± 1·54
Control (8)	110·7 ± 2·8	182·6 ± 4·2	60·68 ± 1·01
Iproniazid (8)† (chronic)	122·9 ± 4·3 P < 0·05	183·0 ± 6·6	67·38 ± 1·86 P < 0·01
Control (8)	106·8 ± 7·7	181·8 ± 9·5	58·46 ± 1·81
Pargyline (8)‡ (acute)	109·9 ± 5·3	175·8 ± 3·2	62·64 ± 2·82
Control (8)	112·6 ± 2·8	194·0 ± 4·0	58·19 ± 1·47
Pargyline (8)§ (chronic)	136·4 ± 3·3 P < 0·001	210·5 ± 3·9	64·84 ± 1·17 P < 0·01

* Rats were injected with a single dose of 100 mg/kg, s.c., and were sacrificed 4 hr later.

† Rats were injected s.c. daily with doses of 100, 50, 50 and 50 mg/kg, the last dose given 4 hr prior to sacrifice.

‡ Rats were injected with a single dose of 100 mg/kg, s.c., and were sacrificed 4 hr later.

§ Rats were given a daily dose of 25 mg/kg, s.c., and were sacrificed 4 hr after the last of the four injections.

TABLE 5. EFFECT OF CERTAIN ANTIDEPRESSANTS AND THEIR INTERACTIONS WITH RESERPINE ON PHOSPHORYLASE LEVEL OF RAT BRAIN

Treatment	−AMP (Mean ± S.E.M.) (phosphorylase units)	+AMP (Mean ± S.E.M.) (phosphorylase units)	$\frac{-AMP}{+AMP} \times 100$ (Mean ± S.E.M.)
Control (8)	114·0 ± 3·6	188·8 ± 4·0	60·23 ± 1·81
Amphetamine (8)*	115·3 ± 4·4	201·4 ± 4·5	57·19 ± 1·51
Control (8)†	113·4 ± 4·0	192·4 ± 3·0	58·96 ± 1·88
Reserpine (8)‡	87·1 ± 4·0 P < 0·001	191·0 ± 3·0	45·53 ± 1·88 P < 0·001
Reserpine + amphetamine (8)§	105·3 ± 4·0 P < 0·01 ‖ P > 0·05	193·0 ± 3·0	54·44 ± 1·88 P < 0·01 ‖ P > 0·05
Pargyline + reserpine (8)¶	124·8 ± 4·0 P < 0·001 ‖	203·6 ± 3·0	61·25 ± 1·88 P ≪ 0·001 ‖

* Rats were injected with a single dose of 10 mg/kg i.p. and were sacrificed 20 min after administration.

† A 4 × 8 randomized complete-block design was used for the three treatment groups below and the control. Vehicle for dissolving reserpine was used as control. See text for explanation of identical S.E.M.

‡ Rats were given a dose of 5 mg/kg i.p. and were sacrificed 3 hr later.

§ Reserpine, 5 mg/kg i.p., was administered at zero hour, followed 3 hr later by amphetamine, 10 mg/kg i.p. Rats were sacrificed usually within 15 min after amphetamine administration. The abrupt change in behavior from deep sedation to hyperactivity occurred early during the period.

‖ Where noted with symbol ‖, the P value is that of comparison to reserpine; otherwise, to control.

¶ Pargyline, 100 mg/kg i.p., was administered at zero hour followed by reserpine, 5 mg/kg i.p., at 1 hr. Rats were sacrificed 4 hr after reserpine.

Table 5 shows the results as well as the details of experimental procedures of such experiments.

The rats given pargyline followed by reserpine were never sedated; in fact, their motor activity was greater than that of control and exophthalmus was pronounced. The phosphorylase activity was somewhat greater than the normal level and was significantly greater than that of the brain of rats given only reserpine. Amphetamine, when given to rats 3 hr after reserpine, reversed the reserpine syndrome. This reversal was abrupt and dramatic. A few minutes after the amphetamine administration, the rat rose suddenly from deep sedation and hunched-back posture and commenced its stereotyped behavior. The phosphorylase level, measured within 15 min of the change in behavior, was significantly greater than that of animals given only reserpine.

Tricyclic antidepressant. Desmethylimipramine (DMI; 20 mg/kg, i.p.) had no effect on the phosphorylase activity by itself 3·5 hr after its administration (Table 6). However, when reserpine (3 mg/kg, i.p.) was injected 0·5 hr after DMI and the animal

TABLE 6. EFFECT OF DESMETHYLIMIPRAMINE (DMI) AND OTHER TRICYCLIC COMPOUNDS ON REDUCTION OF RAT BRAIN PHOSPHORYLASE LEVEL BY RESERPINE

Treatment	−AMP (Mean ± S.E.M.) (phosphorylase units)	+AMP (Mean ± S.E.M.) (phosphorylase units)	$\frac{-AMP}{+AMP} \times 100$ (Mean ± S.E.M.)
Control (6)	113·5 ± 2·4	190·7 ± 3·9	59·67 ± 1·23
DMI (6)*	120·7 ± 3·6	203·3 ± 2·7	59·33 ± 1·35
Reserpine (11)†	89·9 ± 1·8 P < 0·001‡	192·4 ± 3·3	46·78 ± 0·76 P < 0·001‡
DMI + reserpine (12)§	107·5 ± 3·5 P < 0·001‖	202·0 ± 4·5	53·48 ± 2·07 P < 0·01‖
Promazine + reserpine (8)¶	80·3 ± 2·8	176·5 ± 6·1	45·51 ± 1·01
Promethazine + reserpine (8)¶	85·0 ± 3·3	178·8 ± 3·6	47·65 ± 1·92

* Dose was 20 mg/kg i.p.

† Dose was 3 mg/kg i.p. and rats were sacrificed 3 hr later.

‡ The P value is that of comparison to control.

§ DMI, 20 mg/kg i.p., was given at zero hour followed in one-half hr by reserpine, 3 mg/kg i.p. Rats were sacrificed 3 hr after reserpine administration.

‖ The P value is that of comparison to reserpine.

¶ Doses for promazine and promethazine were 20 mg/kg i.p., and the experiments were carried out exactly as for DMI and reserpine.

was sacrificed 3 hr after reserpine, the phosphorylase activity was not at the low level it would have reached if reserpine alone were acting (Table 6). The difference was highly significant. Behaviorally, the difference was small. The rats of the DMI group usually had their eyes slightly open, while those of the reserpine group were tightly closed.

In order to assess whether the effect of a tricyclic antidepressant on the phosphorylase activity is due to its antidepressant property and not to its tricyclic structure, similar experiments were carried out with promazine and promethazine. These drugs are tricyclic in structure, promazine having a depressant effect and promethazine having no effect on the phosphorylase enzyme. In contrast to DMI, these drugs failed to mitigate the effect of reserpine, as may be seen in Table 6.

DISCUSSION

The data presented in this paper show that all tranquilizers* studied, except prochlorperazine, depressed the phosphorylase activity, while the two nontranquilizers, isoreserpine and promethazine, failed to affect it. The results tend to confine phosphorylase activation only to tranquilizers, if adequate explanation is available for the anomaly of prochlorperazine.

Explanation for the anomaly of prochlorperazine may lie in its strong property to induce catalepsy in rats. We have tested a number of phenothiazine drugs for their property to induce catalepsy in rats (a procedure used routinely for screening tranquilizers) and found that phenothiazines of piperazine derivatives such as prochlorperazine and perphenazine were very potent in inducing catalepsy, while promazine, an aliphatic derivative, had no effect and chlorpromazine, another aliphatic derivative, was in between the two (unpublished observations). If we assume that a tranquilizer may possess either one or both of the properties, i.e. to induce catalepsy or to depress the brain phosphorylase activity in rats, it would not be obligatory that prochlorperazine depress the phosphorylase activity. Incidentally, promethazine, another aliphatic derivative but not a tranquilizer, has neither of these properties.

Interpretation of observed phosphorylase activity with antidepressants is difficult because no simple general statement can be made about the effect of these drugs on phosphorylase activity. None of the drugs enhanced the phosphorylase level of normal rats with a single dose. Different types of antidepressants necessitated the use of different procedures to show their effects on the phosphorylase activity. However, if a common action of antidepressants on phosphorylase activity is to be sought, it is that none of them depressed it and all enhanced it whenever it was observed under certain experimental conditions.

The failure of the antidepressants to raise the phosphorylase level of normal rats could be due to refractoriness of the normal level to be enhanced further, or simply it was not meant to be raised, if the drugs' pharmacological actions are any indication. Imipramine, and by implication DMI, causes no excitation in normal human subjects or animals, but elicits its antidepressant action only in depressed patients.[16] Neither iproniazid nor pargyline, when administered alone, caused overt signs of excitation. Such signs were clearly evident when pargyline was followed by reserpine. Amphetamine caused stereotyped behavior, but this excitatory behavior was recently shown to be related to dopamine rather than to norepinephrine,[17,18] and dopamine is not known to be related to phosphorylase activation in any way. Thus, there is interesting circumstantial and experimental evidence to support the latter view.

One cannot be certain that the phosphorylase activity observed in rat brain with tranquilizers and antidepressants is related to the therapeutic property of these drugs. However, if the phosphorylase activation can be explained as a consequence of a reasonable mode of action of these drugs, which may differ from drug to drug, the likelihood of such a relationship will be greatly enhanced. A unified explanation appears possible if one introduces a postulate that links the mode of action with phosphorylase activity.

* The word "tranquilizer" refers to "major" tranquilizers as classified in *Pharmacology and Therapeutics.*[15] A characteristic distinguishing this group of drugs from "minor" tranquilizers as well as from barbiturates and sedatives is that they relieve psychotic symptoms and are employed clinically for the treatment of acute and chronic psychosis.

The postulate is that the phosphorylase activity is enhanced or depressed depending on the availability of norepinephrine at receptor sites, reflecting central adrenergic activity. This postulate is in general agreement with the "catecholamine hypothesis of affective disorders" as reviewed by Schildkraut,[19] if one equates available neurotransmitter at receptor sites with phosphorylase activity.

The postulate is not without foundation. In certain peripheral organs the phosphorylase activity has been found to be enhanced by stimulation of the sympathetic system and depressed by that of the parasympathetic system.[20–22] It even appears that the observed phosphorylase activity is a net result of the sympathetic and parasympathetic activity.[20,22] Such activation of phosphorylase by nerve stimulation may[20] or may not[23,24] involve cyclic AMP, but it is of considerable interest that the brain contains the highest concentration of cyclic AMP of any tissue in the body[25] and that, in a subcelluar study of rat brain by De Robertis *et al.*,[26] cyclic AMP was found in highest concentration in fractions which contained mainly synaptic membranes. However, it should be remembered that increased cyclic AMP content does not constitute *prima facie* evidence of phosphorylase activation because Kakiuchi and Rall[27] observed in cerebral cortex and cerebellar slices no changes in phosphorylase *a* content under conditions producing a large increase in cyclic AMP.

By invoking the above postulate, it is possible to explain the effect of various drugs on phosphorylase activity. In each instance a condition appears to prevail that would have an effect on norepinephrine concentration at receptor sites and hence on phosphorylase activity.

Reserpine depletes norepinephrine content.[28] Chlorpromazine and presumably most phenothiazine tranquilizers are thought to cause tranquilization, at least in part, by adrenergic block.[13] Desmethylimipramine decreases membrane permeability and prevents inactivation of released norepinephrine by re-uptake mechanism.[29] Monoamine oxidase inhibitors tend to increase the intraneuronal concentration of norepinephrine by preventing inactivation of the amine. Reserpine, when administered after pargyline, releases the accumulated norepinephrine.[30] Amphetamine acts by releasing endogenous adrenergic transmitters.[31]

The fact that it is possible to explain the effect of tranquilizers and antidepressants on the phosphorylase activity of rat brain on the basis of their mode of action and a postulate that involves adrenergic receptors suggests strongly that the phosphorylase activity may be a barometer for CNS adrenergic activity.

In spite of the apparent importance of the phosphorylase in brain function, two facts tend to counteract it. The first is the smallness of the change in phosphorylase activity observed with various drugs, although the change is statistically significant. We do not know in which part of the brain the changes due to drugs are taking place. It is entirely possible that some areas of the brain would respond to drugs more than others. Since we used whole brain for assay, we could only determine the average change in the whole brain. Perhaps studies of the effect of drugs on discrete areas of the brain would solve the problem. The second is that the glycogen content of brain is known to be very small, and moreover, the K_m value of glycogen phosphorylase *a* is relatively large compared to that of muscle.[32] This would tend to throw doubt on the biological significance of brain phosphorylase. However, the K_m decreases when inorganic phosphate or AMP concentration is increased[32] so that *in vivo* it may not be as large as it appears. Also, recently Drummond and Bellward[33] studied the

distribution of phosphorylase kinase, an enzyme that converts phosphorylase from its inactive form to the active form, in neural tissues of different animals and found it to be highest in the brain and in two ganglia, both rich in synapses. They suggested that glycogenolysis may be coupled in some way with synaptic transmission. Since synapses constitute only a small portion of neural tissue, available glycogen may be sufficient for their need. Incidentally, their suggestion is in complete accord with our view.

In conclusion, the results of this investigation suggest that the glycogen phosphorylase of brain may be involved in the actions of many drugs used as tranquilizers and antidepressants.

Acknowledgements—We wish to thank the following companies for generously supplying us with the drugs used in this study: Schering Corp. for perphenazine, E. R. Squibb & Sons for triflupromazine, Smith, Kline & French Laboratories for prochlorperazine ethanedisulfonate, Wyeth Laboratories for promazine hydrochloride and promethazine hydrochloride, Hoffmann-LaRoche for iproniazid phosphate, Abbott Laboratories for pargyline hydrochloride, Geigy Pharmaceuticals for desmethylimipramine, and Ciba Pharmaceutical Company for 3-isoreserpine.

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