tion of flavonoids with the NADPH-oxidase, these studies demonstrate that inhibition of the human neutrophil respiratory burst is highly specific in regard to flavonoid structure, and this effect may be related to differing permeability characteristics. These compounds may be suitable agents for further study of the sequence of activation events to enzyme expression in this system. More broadly, the anti-inflammatory activity of the flavonoids has been observed in a variety of model systems [14] and recently extended to inhibitory activity against effector cells of the inflammatory response [5–7, 12, 15]. The structural basis for this anti-inflammatory activity has potential therapeutic importance.

In summary, the generation of the respiratory burst of the human neutrophil is dependent on intact receptor–ligand activation pathways and a flavin-dependent NADPH-oxidase. Flavonoids, a class of phenolic plant pigments, exhibit a high degree of structural specificity (differing in position or number of hydroxyl constituents) in their inhibitory effects on this system. The flavonoids quercetin, fisetin and kaempferol exhibited comparable IC_{50} values for inhibition of oxygen consumption (*ca.* 100 μM) while the structurally similar flavonol, morin, had no significant effect on whole cell oxygen metabolism, but exhibited an identical inhibitory profile to that of quercetin on the NADPH-oxidase. The ability of a particular flavonol to inhibit the respiratory burst in intact cells paralleled its hydrophobicity as measured by partition in octanol and saline. These studies demonstrate highly specific structural requirements of flavonoid inhibitory activity of the human neutrophil respiratory burst and suggest that permeability characteristics of the flavonols might determine their inhibitory activity in intact cells.

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* Reprint requests should be addressed to: Alfred I. Tauber, M.D., Boston City Hospital, Boston, MA 02118.
† Current address: SRI International, Menlo Park, CA 94025.

Departments of Medicine and Biochemistry ALFRED I. TAUBER*
Boston University School of Medicine, JUDITH R. FAY†
Department of Medicine MICHAEL A. MARLETTA
Boston City Hospital
Boston, MA 02118, U.S.A., and
Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge, MA 02139, U.S.A.

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Depressive action of γ-aminobutyraldehyde as a precursor of γ-aminobutyric acid

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The inhibitory role of γ-aminobutyric acid (GABA) in central nervous system mechanisms is well known. Recent evidence indicates a biochemical relation between the functioning GABA system and the symptoms of Huntington's disease [1], Parkinsonism [2], and epilepsy [3]. Since GABA itself can scarcely penetrate the blood–brain barrier [4], agents have been sought which act on the GABA system and thus have the potential for altering the course of these neurological disorders [5, 6]. A major difficulty in the design of therapeutic agents acting through the GABA system is the poor brain-penetrating properties of active compounds.

A recent study in this laboratory has shown the presence in mammalian brain of an enzyme that can catalyze oxidation of 4-aminobutyraldehyde (ABAL) to GABA [7]. Subsequently, it has been reported that peripherally administered ABAL can easily penetrate the blood–brain barrier into the brain and is rapidly oxidized there to GABA [8, 9].

On the other hand, the problem of the relationship between the increase in brain GABA level and the changes in behavior of animals is still open to dispute, both as a problem of therapy and as a basic biochemical question on the role of GABA. This paper describes the depressive

action of peripherally given ABAL on the spontaneous motor activity of mice and the relationship between the depressive action and the production of GABA in brain from the ABAL.

Materials and methods

[^{3}H]ABAL and unlabeled ABAL were prepared from [^{3}H]putrescine (New England Nuclear) and 4-aminobutyraldehyde diethylacetal (Aldrich Chemical Co.), respectively, by the method previously reported [7–9]. [^{3}H]ABAL or unlabeled ABAL was dissolved in saline, and the final concentration of the solutions for administration was adjusted to 100 mM. Ten microliters of the solutions per g body weight was subcutaneously injected in male DDY mice (about 20 g). For oral administration, 20 μl of the solutions per g body weight was given through a cannula into the gastric cavity.

Mice that had been given [^{3}H]ABAL were decapitated at adequate intervals after the treatments. [^{3}H]ABAL, [^{3}H]GABA and the deaminated metabolites of [^{3}H]ABAL present in brain were measured using the method previously described [7–9]. The brains were homogenized with 4 vol. of cold 1 M perchloric acid and centrifuged. The extract was adjusted to pH 4 with KOH, centrifuged, and applied to a column of Dowex 50-H$^+$ (7 × 35 mm). The column was washed once with 20 ml of water, and the effluent (pass fraction) was used to assay deaminated metabolites of [^{3}H]ABAL. ^{3}H-Amino acids and [^{3}H]ABAL were eluted from the resin bed with 12 ml of 2 M NH_4OH, and the radioactivity of the eluate (NH_4OH fraction) was measured in a scintillation spectrometer. A portion of the eluate was evaporated to dryness under vacuum; [^{3}H]ABAL was eliminated completely from the eluate after this procedure. The residue was redissolved in an adequate volume of water, and an aliquot of this (amino acid fraction) was also counted to estimate radioactive amino acids. The radioactivity of [^{3}H]ABAL was calculated by subtracting the radioactivity of the amino acid fraction from that of the NH_4OH fraction. The radioactivity of [^{3}H]GABA was determined by paper chromatographic analyses of the amino acid fraction [9].

Spontaneous motor activity of mice was observed by

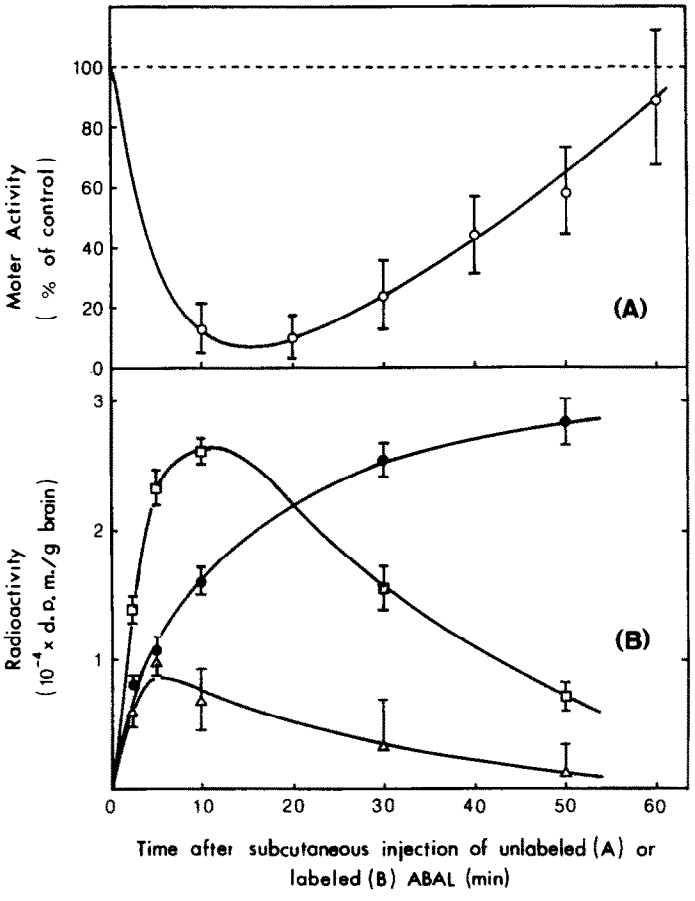

Fig. 1. Time courses of the GABA production in brain from ABAL injected subcutaneously and of the motor activity of mice after the treatment. Key: (○—○) motor activity; (□—□) [^{3}H]GABA; (△—△) [^{3}H]ABAL; and (●—●) deaminated metabolites of [^{3}H]GABA. Following subcutaneous injection of ABAL (1 μmole/g body weight), the motor activity was monitored by measuring the number of revolutions of the revolving activity cage [10], and the change of the motor activity was expressed as a percentage of the number of revolutions of control mice which received the same volume of saline. Each value represents the mean ± S.E.M. for ten mice. The number of revolutions per 10 min of control mice was 112 ± 12 (mean of ten mice ± S.E.M.). Following subcutaneous injection of [^{3}H]ABAL (34 nCi/μmole/g body wt), [^{3}H]ABAL, [^{3}H]GABA and its deaminated metabolites in brain were extracted and estimated by the method previously described [7–9]. These amounts were expressed as nmoles/g brain which was calculated from these radioactivities. The values are the mean ± S.D. for three experiments.

means of a revolving activity cage [10]. The number of revolutions was recorded continuously by a digital meter. The mice were left in each cage for about 20 min, and then they received, subcutaneously or orally, unlabeled ABAL. Control animals received the same volumes of saline by the same procedures. Immediately after the administrations, the number of revolutions per 10 min was estimated sequentially. Changes of the motor activity were expressed as percent of the number of revolutions of control mice, which was 112 ± 12 per 10 min (the mean of ten mice ± S.E.M.). The experiments were carried out in a room thermoregulated at 20°.

Results and discussion

ABAL significantly inhibited the spontaneous motor activity following both subcutaneous (1 μmole/g) and oral (2 μmoles/g) administration, though the effects were somewhat different between both treatments (Figs. 1 and 2). Immediately following the subcutaneous injection, the motor activity fell rapidly and reached a minimum at 10–15 min. In contrast, the oral administration produced a slow developing inhibition of the motor activity, and the inhibition continued for a longer time.

The overall correlation between the changes in the motor activity and the conversion of [^{3}H]ABAL in brain to [^{3}H]GABA was investigated (Figs. 1 and 2). Following the subcutaneous injection of [^{3}H]ABAL (1 μmole, 34 nCi/g body wt), the levels of [^{3}H]ABAL in the brain increased in the first 5 min and then gradually decreased, while following the oral administration of [^{3}H]ABAL (2 μmoles, 68 nCi/g) [^{3}H]ABAL was hardly found in the brain, probably due to its conversion to [^{3}H]GABA. The [^{3}H]GABA levels increased rapidly following the injection of [^{3}H]ABAL and reached a maximum at about 10 min. In contrast, the oral administration of [^{3}H]ABAL produced a slow increase in the [^{3}H]GABA levels, which reached a maximum at about 30 min. The radioactivities of deaminated metabolites of [^{3}H]GABA increased progressively with time following both administrations of [^{3}H]ABAL.

The inhibition of motor activity induced by the ABAL administration began with the production of [^{3}H]GABA in brain from [^{3}H]ABAL, continued with the maintenance of [^{3}H]GABA, and terminated with the disappearance of [^{3}H]GABA, as shown in Figs. 1 and 2. If the turnover of GABA in brain is not affected by the ABAL administrations, the GABA levels in brain should be increased by the treatments; the concentration of [^{3}H]GABA is calculated as about 350 nmoles/g brain 10 min after the [^{3}H]ABAL injection and as about 530 nmoles/g brain 30 min after the oral administration of [^{3}H]ABAL respectively. Therefore, the above correlations between the motor activities and the brain [^{3}H]GABA levels suggest that the inhibition of motor activity induced by ABAL treatment may be caused by an increase in the brain GABA levels.

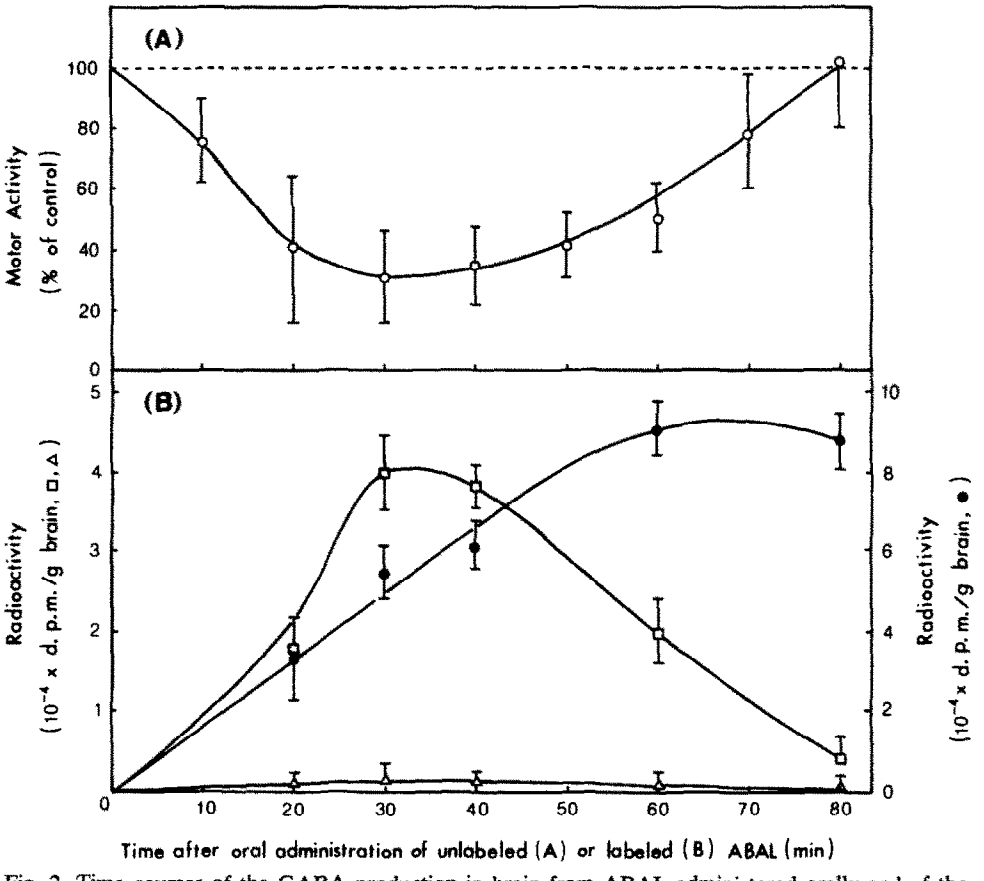

Fig. 2. Time courses of the GABA production in brain from ABAL administered orally and of the motor activity of animals after the treatment. Key: (○—○) motor activity; (□—□) [^{3}H]GABA; (△—△) [^{3}H]ABAL; and (●—●) deaminated metabolites of [^{3}H]GABA. Following oral administration of ABAL (2 μmoles/g body weight), the motor activity was measured according to the method described in the text and in the legend of Fig. 1. The activity is expressed as a percentage of that of control mice, and each value is the mean ± S.E.M. for ten mice. [^{3}H]ABAL, [^{3}H]GABA and its deaminated metabolites in brain following oral administration of [^{3}H]ABAL (68 nCi/2 μmoles/g body weight) were assayed according to the procedure described in the text and Fig. 1. Each value represents the mean ± S.D. for three experiments.

In summary, we have reported here that ABAL had a depressive effect on the motor activity of animals, and that the effect may be associated with the production of GABA from ABAL in brain. ABAL may serve as a therapeutic prodrug of GABA for some neurological disorders of the GABA system, though the problem of whether the physiological actions of GABA produced from ABAL are exactly equal to those of endogenous GABA has yet to be investigated thoroughly.

Department of Biochemistry MAKOTO MATSUDA*
The Jikei University School of TETSUO KUWAHARA
Medicine MASAKAZU SUGAHARA
Tokyo 105, Japan

* Address all correspondence to: Professor Makoto Matsuda, Department of Biochemistry, The Jikei University School of Medicine, 3-25-8, Nishi-shinbashi, Minato-ku, Tokyo 105, Japan.

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Increase in serum corticosterone concentration and decrease in hypothalamic epinephrine concentration by *N*-propylnorapomorphine in rats

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N-n-Propylnorapomorphine (NPA) has been described to be a dopamine agonist with greater potency and slightly longer duration of action than apomorphine [1]. NPA has been shown to decrease dopamine turnover in brain and to reduce the firing rate of nigral dopamine neurons [2], to cause turning in rats with unilateral nigrostriatal lesions [3], to cause hypoactivity at low doses and hyperactivity at higher doses [2, 4], and to cause stereotypy in rats and mice [4, 5]. Recently we have described two additional effects of dopamine agonists in rats, namely elevation of serum corticosterone concentration [6] and lowering of hypothalamic epinephrine concentration [7]. Since neither of these effects has been reported for NPA, and since most of the dopamine agonists we studied previously were ergolines, we determined whether NPA had the ability to increase serum corticosterone concentration and decrease hypothalamic epinephrine concentration in rats.

Male Wistar rats weighing 150–200 g (from Harlan Industries, Cumberland, IN) were housed in groups of five in a 24° room with 12 hr light:dark cycles for at least 1 week prior to the experiment. All rats had free access to food and water during the experiments. Drugs were injected i.p. The injection volume was 1 ml/kg, control rats receiving vehicle at the same volume. (−)NPA hydrochloride (Research Biochemicals, Wayland, MA) was dissolved in distilled water, and spiperone (Janssen Pharmaceutica, Beerse, Belgium) was dissolved in 0.01 N HCl. Treated rats were decapitated, and blood collected from the trunk was allowed to clot. Serum obtained after centrifugation was stored at −15° prior to analysis. Whole brain or dissected brain regions were frozen on dry ice and stored at −15° prior to analysis. Corticosterone was measured spectrofluorometrically [8]. High performance liquid chromatography with electrochemical detection was used to measure epinephrine [9] and the dopamine metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) [10]. All results are shown as mean values ± standard errors for five rats per group. Comparisons between groups were made by Student's *t*-test.

NPA increased serum corticosterone concentration and decreased DOPAC and HVA concentrations in whole brain (Table 1). The 0.003 mg/kg dose had no significant effect, but 0.1 and 0.3 mg/kg doses increased corticosterone dose-dependently, the 1 mg/kg dose causing no further increase. DOPAC and HVA concentrations were decreased significantly at the 0.1 to 1 mg/kg doses of NPA.

Spiperone pretreatment increased whole brain concentrations of DOPAC and HVA but had no significant effect on serum corticosterone (Table 2). In spiperone-pretreated rats, the increase in serum corticosterone and the decrease in cerebral DOPAC and HVA caused by NPA in control rats were blocked completely.

Epinephrine concentration in hypothalamus was decreased significantly by NPA at a dose (1 mg/kg) that decreased striatal dopamine metabolites (Table 3). Spiperone pretreatment overrode the decrease in striatal DOPAC and HVA and prevented the decrease in hypothalamic epinephrine.

These findings reveal that NPA, like other dopamine agonists we have studied, decreased hypothalamic epinephrine [7] and increased serum corticosterone [6, 11]. That these actions of NPA were mediated by dopamine receptor activation is supported by the findings that these changes (1) occurred at NPA doses that decreased whole