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THE LOCALIZATION OF A NON-TRANQUILIZER PHENOTHIAZINE IN THE DOG CEREBELLUM AND ASSOCIATED AREAS*

P. S. GUTH and J. AMARO

Department of Pharmacology, Tulane University School of Medicine, New Orleans, La., U.S.A.

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Abstract—It was shown earlier that thiethylperazine (TPZ), a potent antiemetic, antinauseant drug, exhibited a markedly different spectrum of localization in dog brain from the tranquilizer phenothiazines, chlorpromazine and prochlorperazine. TPZ's localization in the cerebellar areas was particularly striking. A closer inquiry into the localization of TPZ among cerebellar areas has been made in an investigation of the possible correlation between sites of action and concentration among phenothiazines. Qualitative and quantitative determinations of the presence of the drug in lingula, flocculus, nodulus, uvula, posterior cortex and fastigial nucleus, as well as in restiform body and area of the vestibular nuclei, was made 5, 15, 30, 45, 60, and 90 min after i.v. administration of the drug. The flocculus and nodulus never demonstrated detectable drug. The cerebellar cortex and lingula exhibited low concentrations, and the fastigial nucleus, restiform body, and vestibular nuclear area exhibited highest concentrations. The three last-named areas are generally concerned with cerebellovestibular fibers, and this may underline the mechanism of action of this drug.

IT WAS previously shown[1] that the tranquilizer phenothiazines, chlorpromazine and prochlorperazine, concentrated in dog brain differently from the non-tranquilizer phenothiazine, thiethylperazine (TPZ).† A high degree of correlation existed between the areas of concentration of the tranquilizers and those areas judged to be the major sites of action from electropharmacologic evidence.[2] In the study of de Jaramillo and Guth,[1] the areas exhibiting the highest concentrations of TPZ were in the cerebellum. However, only vermis and paraflocculus were studied at that time. It was therefore decided to enlarge the study of TPZ distribution among cerebellar areas, particularly those concerned with vestibular function, to determine the sites of concentration for TPZ, so that in later studies correlations between sites of action and concentration may be evaluated.

METHOD

Mongrel dogs of both sexes, 10–15 kg were used. At 5, 15, 30, 45, 60, and 90 min after the i.v. injection of 10 mg TPZ as the base/kg, the dogs were sacrificed by intravenous air injection.‡ The head was perfused with 1 liter of 0·1 M phosphate buffer

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† Torecan, Sandoz; 2-(ethylthio)-10-[3-(4-methyl-1-piperazinyl)-propyl]-phenothiazine.

‡ In several dogs, electrocution was tried as the method of sacrifice. This produced the interesting phenomenon of a high tissue blank fluorescence and so was discarded.

(pH 7·4), 500 ml through each common carotid to remove blood. Outflow was through the severed jugular vein. Chromatography of the perfusate showed no evidence of the injected drug.

The brain was then removed intact. Portions of the cerebellum and other areas were taken as described below. Each area was weighed. The areas were homogenized in 2·0 ml of 0·1 M phosphate buffer (pH 7·4) in glass hand homogenizers. Additional buffer to make a final of 4·0 ml was used to rinse the homogenizer.

The homogenates and rinsings were placed in a test tube, and 1 ml of 12 N HCl was added. The test tube was then placed in a boiling-water bath for 8 min. After cooling at room temperature and centrifuging at 1,400 *g* for 20 min, the supernatant fluid was decanted. The fluorescence of the extract was measured in a Turner fluorometer; Corning 7-60 (peak 350 mμ, range 290–400 mμ and Kodak-8 (passes wavelengths above 485 mμ) were used as activation and emission filters respectively.

The pH of the extract was then adjusted to 9·0 and the drug extracted into two portions of ethylene dichloride–10% isoamyl alcohol (each portion 2–5 times the starting volume) by shaking for 15 min each time. The solvent mixture was evaporated to dryness and the residue dissolved in methanol. The methanol solution of the drug was further partially evaporated. Finally, the methanol solution was spotted on thin-layer (0·25 mm thick) alumina activated for 1 hr at 120°. The plate was run in chloroform: propanol (100:1) and developed with a spray of concentrated sulfuric acid.

The efficiency of the extraction procedure (drug recovery) was determined by incubating fresh homogenates of each cerebellar area to be extracted with known amounts of drug for 10 min at 37°. That the tissue had absorbed all the added drug was determined chromatographically by observing that the supernatant fraction of the incubation mixture showed no drug.

The average fluorescence of blank extracts of each of the regions of four uninjected dogs was subtracted from the values obtained in the treated dogs.

Cerebellar areas studied were: lingula, flocculus, nodulus, uvula, area of the fastigial nucleus, and several portions of posterior cortex; the restiform body and area of vestibular nuclei were also investigated. In general, 100 mg of all areas except vestibular nuclei and cerebellar cortex were obtainable. One gram of cortex and 50 mg of vestibular nuclei were the usual weights of these areas.

RESULTS

Drug concentrations

In Fig 1, the presence of TPZ in the areas and at the times studied is indicated by either an asterisk or a vertical bar. The asterisk indicates that, although the drug was present as witnessed by chromatographic evidence, the probability value calculated by the "t" test was greater than approximately 0·075. These high values arose as a result of the very high tissue blank fluorescence and the relative insensitivity of the method. In general, the asterisks indicate a concentration of not less than 20 ug/g tissue, as determined from the minimal detectable amount on the chromatogram. Where P values less than 0·075 were obtained, the actual calculated tissue concentrations are given as vertical bars. Again, the presence of the drug in the extract was always confirmed chromatographically. The area of vestibular nuclei showed highest concentrations, followed by fastigial nucleus and cerebellar cortex. The restiform

body likewise gave rather consistent evidence of drug, whereas, nodulus flocculus, and uvula never exhibited drug, and the lingula showed drug only at one sampling time.

Recovery values

The recovery values of TPZ added *in vitro* were similar for all eight tissue areas studied. These values averaged 95% (range, 90–110%).

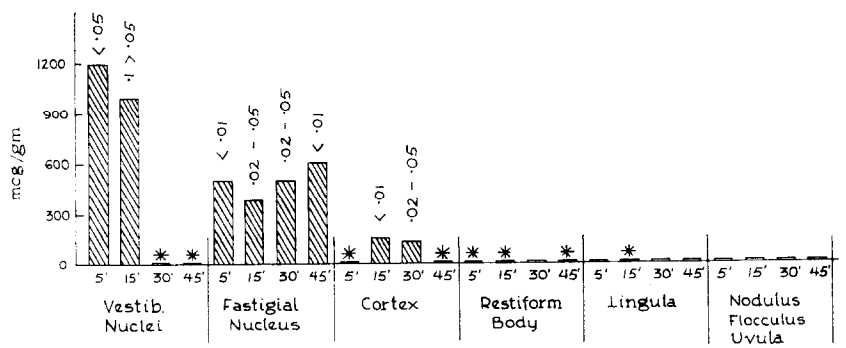

FIG. 1. Thiethylperazine distribution in dog cerebellum. Numbers on the ordinate refer to times after injection in minutes. Asterisks indicate that the drug was visualized on chromatograms of extracts but that the P values produced by "t" test were greater than 0·075. The more significant P values are given over each bar; 60- and 90-min values are omitted because of the consistent presence of metabolites on chromatograms.

Chromatography

Our chromatographic procedure permitted separation of TPZ from all metabolites studied. Only the disulfoxide and disulfone could not be clearly distinguished from each other. The following are the R_f values and colors obtained with sulfuric acid spray; TPZ, aqua, 0·93; TPZ–sulfoxide side chain, pink, 0·52; TPZ–sulfoxide ring, violet, 0·65; TPZ–disulfoxide, rust, 0·42; TPZ–disulfone, rust, 0·40.

There was consistent evidence of metabolites of TPZ beyond the 45-min sampling time. Therefore the 60- and 90-min results were not included in Fig. 1.

The chromatograms provided qualitative confirmation of the presence of the drug. Beyond this, chromatography established a lower limit for the tissue concentration of the drug. That is, spots containing less than 2 μg TPZ could not be visualized. When the chromatograms indicated the presence of metabolites in a few instances earlier than 45 min, these results were not included in Fig. 1.

DISCUSSION

In our earlier work[1] the physical localization of the tranquilizer drugs, chlorpromazine and prochlorperazine, was found to correlate well with areas thought to be sites of actions as judged from electropharmacologic evidence.[2] In the present study the tested drug TPZ, has no such relatively well-defined sites of action. Therefore

the results presented here, while of interest in themselves, represent only half of a study attempting to correlate sites of action and localization. Now that the areas of concentration of TPZ in cerebellum and associated tissue have been delineated it remains to destroy these areas and determine whether the drug still retains its characteristic pharmacologic activity. In preliminary studies, using totally cerebellectomized cats and a few patients with cerebellar degeneration, the drug seemed less effective than in normal cats and humans.

In an extended series of experiments reviewed by Tyler and Bard,[3] the integrity of the flocculonodular lobe of the cerebellum of the dog was found to be necessary for the development of motion sickness. In the present experiments the drug did not, however, localize in either flocculus or nodulus. The areas showing highest concentrations (vestibular nuclei, fastigial nucleus, cerebellar cortex, and restiform body) are, in general, involved with cerebellovestibular fibers, whereas areas involved with vestibulocerebellar tracts[4] were not shown to contain the drug.

It is perhaps important to note that the time of consistent appearance of metabolites in the chromatograms made of the extracts coincided with the terminations of drug action, as determined in dogs by means of rotationally-induced nystagmus as the test procedure.*

It should be pointed out that certain areas of the cerebullum showed drug concentrations of the order of 10^{-3} M. In the past, *in-vitro* studies employing phenothiazines have been criticized on the basis that the drug concentrations used were excessive. Since tissue levels of 10^{-3} M have now been shown to exist, these criticisms are no longer applicable, at least in cerebullar tissue. It should also be noted that a mitochondrion within a cell of a tissue containing 10^{-3} M may have an even higher local concentration. That is, that the drug need not be distributed evenly among all the cell structures, and that some structures will therefore have less and some more than the average concentration. For example, Berger[5] and Guth[7] have demonstrated the strong affinity of brain mitochondria for phenothiazines.

A study of the distribution of TPZ among cerebellar subcellular organelles is under way.

This paper is one of a series designed to test the hypothesis that, as a group, the phenothiazines concentrate at their sites of action and affect membrane permeability and therefore the function of organelles within areas of high concentration. In other words, that the phenothiazines are capable of affecting membrane permeability in general, and wherever physical, chemical, and physiological factors dictate that they concentrate, membranes within these areas are affected.

Thus far it has been demonstrated that the permeability of membranes of mitochondria,[6] pinched-off nerve endings,[8] red blood cells[9], and lysosomes[10] are influenced by the presence of phenothiazines. It has also been demonstrated that chlorpromazine and prochlorperazine concentrate at areas deemed to be their sites of action! Therefore, it is conceivable that medically useful actions are produced by the fortuitous concentration of phenothiazines in certain nervous system areas, and that this concentration leads to altered function. The work in the present paper taken in the light of the above considerations engenders the working hypothesis that the site of action of the antiemetic phenothiazine, thiethylperazine, is among cerebello vestibular areas.

* W. Rubin and G. Ratcliff, unpublished observations.

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