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PRODUCTION OF CATALEPSY AND DEPLETION OF BRAIN MONOAMINES BY A BUTYROPHENONE DERIVATIVE

LUIS D. FUENMAYOR[1] & MARTHE VOGT

A.R.C. Institute of Animal Physiology, Babraham, Cambridge CB2 4AT

1 The cataleptic and monoamine-depleting effects of a butyrophenone derivative (4'-fluoro-4-[[4-(*p*-fluorophenyl)-3-cyclohexen-1-yl]-amino]-butyrophenone hydrochloride, U-32, 802A) were studied in rats and mice and compared with those of tetrabenazine.

2 Catalepsy was evaluated by means of a modified grid test which allowed the repetition of the test in the same animal several times without affecting the results. Both drugs produced a dose-related cataleptic state of similar time course.

3 Like tetrabenazine, U-32, 802A induced a large reduction in the content of 5-hydroxytryptamine, dopamine and noradrenaline in different parts of the brain, with a concomitant elevation in the metabolites 5-hydroxyindol-3-yl acetic acid and homovanillic acid. The time courses of the catalepsy and the reduction in brain monoamines were very similar.

4 The activity of U-32, 802A suggested that the drug, although chemically a butyrophenone, might act primarily at the presynaptic organelle for storage of monoamines in a way similar to tetrabenazine.

Introduction

Reserpine and related compounds produce large reductions in the concentration of brain monoamines. On the other hand, neither phenothiazines nor butyrophenones cause a depletion of monoamines from the brain unless used in high doses. However, both chlorpromazine and haloperidol, the prototypes of phenothiazines and butyrophenones, modify the metabolism of brain monoamines. Carlsson & Lindqvist (1963) showed that the accumulation in brain of methoxytyramine and normetanephrine, which occurs after treatment with a monoamine oxidase inhibitor, was enhanced by these drugs. Later, both drugs were shown to increase the concentration of homovanillic acid (HVA) and of dihydroxyphenylacetic acid, the two major metabolites of dopamine, in the brain of different animal species (Andén, Roos & Werdinius, 1964; Bernheimer & Hornykiewicz, 1965; Laverty & Sharman, 1965; Sharman, 1966; Roffler-Tarlov, Sharman & Tegerdine, 1971; Gérardy & Cajgfinger, 1972). Whereas the depletion of amines and the increase in amine metabolites elicited by reserpine depend on a selective blockade of the specific mechanism for incorporating monoamines into the storage granules (Carlsson, Hillarp & Waldeck, 1963), the rise in catecholamine metabolites after chlorpromazine or haloperidol seems to be due to an activation of the catecholaminergic neurones in response to a blockade by these drugs of the receptors on the effector cells (Carlsson & Lindqvist, 1963; Sharman, 1966).

In 1974, Lahti & Lednicer described the butyrophenone derivative 4'-fluoro-4-[[4-(*p*-fluorophenyl)-3-cyclohexen-1-yl]-amino]-butyrophenone hydrochloride (U-32, 802A) as a compound intermediate between butyrophenones and reserpine, with strong depleting effect on brain catecholamines and only a mild action on 5-hydroxytryptamine (5-HT). The drug was also found to release noradrenaline (NA) from the mouse heart, to block the uptake of [^{3}H]-NA into the same tissue, and to diminish the uptake of [^{14}C]-5-HT into the spleen of the mouse. U-32, 802A has since been shown to induce catalepsy in experimental animals (Fuenmayor & Vogt, 1977). This paper describes the changes in monoamine concentrations and metabolism induced in the brain of rats and mice by the administration of U-32, 802A, and their relation to the cataleptogenic effect of the drug. The actions of tetrabenazine, a benzoquinolizine derivative with monoamine depleting properties (Pletscher, 1957) and cataleptogenic activity, was investigated for comparison. Tetrabenazine was chosen instead of reserpine, because the duration of its action resembled that of U-32, 802A.

[1] Present address: Universidad Central de Venezuela, Cátedra de Farmacologia, Escuela de Medicina 'José M. Vargas', Apartado de Correos 76359, Caracas 10, Venezuela.

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Methods

Animals

Adult male albino rats (180 to 260 g), obtained from the breeding colony of the Institute (Wistar-derived strain), and adult male albino mice (Tuck's No. 1, 20 to 30 g) were used. The animals were kept in groups of 8 to 15 with free access to food and water and under controlled day-light with white light on from 05 h 00 min to 19 h 00 min and off from 19 h 00 min to 05 h 00 min. Some of the mice were kept in reversed daylight, a red light being on from 10 h 00 min to 22 h 00 min. Unless stated otherwise, the experiments were carried out during the light period and catalepsy observations or killing of the animals for neurochemical studies were never performed before 07 h 00 min.

Evaluation of catalepsy

Preliminary experiments suggested that a slightly modified vertical grid test (Simon, Malatray & Boissier, 1970) would be more reliable and easier to carry out than other tests. The rat or mouse was gently placed on a vertical (0.64 mm^2 mesh) wire grid measuring 49 × 35 cm. Catalepsy was considered to be present only when the animal remained completely immobile on the grid, whereas Simon *et al.* (1970) based their test on the immobility of the animal's paws and disregarded head movements. The intensity of the cataleptic state was assessed by measuring the duration of the longest immobile episode within a 2 min observation period. If the animal was still immobile at the end of 2 min, it was observed till the first movement occurred; usually the immobility finished with a movement of the animal's head. When an animal came off the grid before the end of 2 min, it was again placed on the grid and observed. This procedure was repeated if necessary until the 2 min observation period was up. The longest time the animal remained in total immobility was measured with a stopwatch at the precision of 0.2 s and expressed in min.

The animals were brought into the testing room (19 to 22°C) at least 1 h before the beginning of the experiment in order to allow adaptation to the new environment. The testing procedure was carried out in the presence of the experimenter only. In the time course experiments, rats were tested before, as well as 0.5, 1, 2, 4, 6, 8 and 24 h after the injection of the drug or vehicle; these experiments were started between 07 h 00 min and 09 h 30 min. In the other experiments animals were tested before, and 2 h after the injection of the drug or vehicle. The whole experiment was performed between 13 h 00 min and 16 h 00 min. Each experiment included animals treated in different ways and, at least, one untreated or vehicle-treated animal.

The statistical analysis was carried out by the two-sample rank test developed by Mann & Whitney (Goldstein, 1964). Differences between means were considered to be statistically significant when the value of P was less than 5% ($P < 0.05$) . As with any other non-parametric test, the two-sample rank test makes no assumption about the normality of a population distribution (Goldstein, 1964).

Chemical estimations

Animals were decapitated at different times after drug administration, the brain removed in less than 1 min and placed in a Petri dish resting on ice. After dissection, tissue samples were put into small glass vials, weighed to a precision of 0.1 mg and the vials stored in ice. Fluorimetric estimations were carried out on the same day.

For the estimation of dopamine and HVA the forebrain was divided along the midline, the lateral ventricles were opened and both striata dissected out by following the borders of the striatum (caudate-putamen) with sharp pointed forceps. The whole procedure was performed within 4 min of killing. Dopamine was estimated in tissue from one animal by the method of Laverty & Sharman (1965), while HVA was estimated in the pooled striata from 2 to 3 animals by the method of Murphy, Robinson & Sharman (1969).

In the mouse, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were estimated in the forebrain and in the combined medulla, pons and midbrain. The forebrain was separated from the midbrain by a section extending from the rostral border of the superior colliculi to the posterior edge of the hypothalamus. In the rat, 5-HT and 5-HIAA were estimated in the forebrain (dissection as in the mouse), the midbrain and the combined pons-medulla. The cerebellum and the pineal gland were always discarded. The dissection was carried out within 3 min of killing. The fluorimetric estimation was carried out by the methods of Bertler (1961) and Contractor (1966) as described by Ahtee, Sharman & Vogt (1970).

NA estimations were carried out in the rat in combined hypothalamus, midbrain and pons-medulla, by the method described by Sharman (1971).

The biochemical data were subjected to the two-tailed Student's t test for non-paired samples (Goldstein, 1964). A probability level of $P < 0.05$ was considered as indicative that the samples were not from the same population.

Drug treatment

U-32, 802A (The Upjohn Company) was dissolved in 0.75% tartaric acid and the pH was adjusted to 7

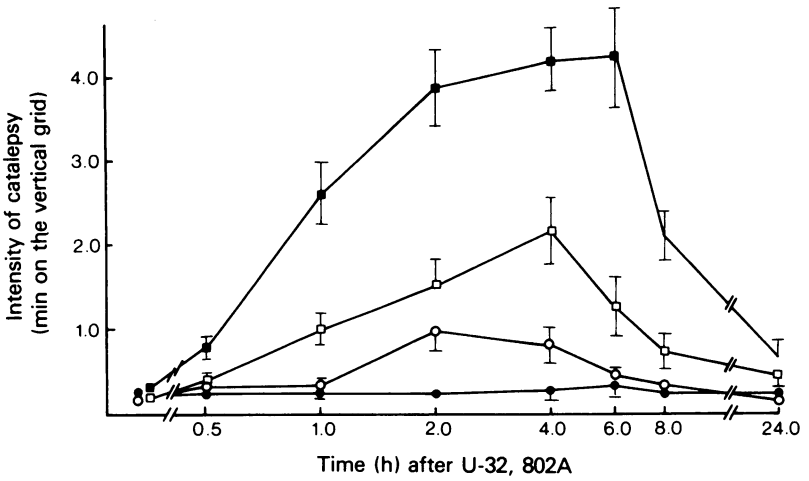

Figure 1 Time course of the catalepsy produced by subcutaneous administration to rats of U-32, 802A: (○) 1 mg/kg; (□) 2 mg/kg; (■) 5 mg/kg. The control group (●) included untreated and vehicle-treated animals. Symbols are means and vertical bars represent s.e. mean of about 12 experiments. Ordinates: duration of immobility on the grid (min). Abscissae: time (h) after injection.

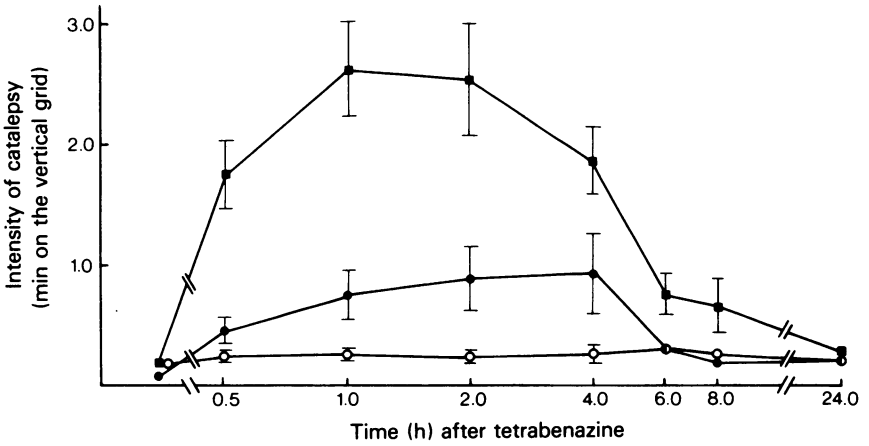

Figure 2 Time-course of the catalepsy produced by subcutaneous administration to rats of tetrabenazine, 2 mg/kg (●) or 4 mg/kg (■). The control group (○) included untreated and vehicle-treated animals. Symbols are means and vertical bars represent s.e. mean of at least 12 experiments. Ordinates: duration of immobility on the grid (min). Abscissae: time (h) after injection.

with sodium hydrogen carbonate immediately before subcutaneous administration. In the rat, doses of 1, 2 or 5 mg/kg were used at a concentration of 1 mg/ml. Tetrabenazine (Roche Products Ltd.) was dissolved in 0.05 N HCl to make a 0.1% solution. The pH was adjusted to 4 with sodium hydrogen carbonate before administration by subcutaneous injection. Rats received 2 or 4 mg/kg, mice 10 or 15 mg/kg.

Results

Normal and cataleptic behaviour on the grid

When control animals were placed on the vertical grid for the first time, they did not stay on the spot on which they were placed, but immediately moved away and very often came off the grid. When the

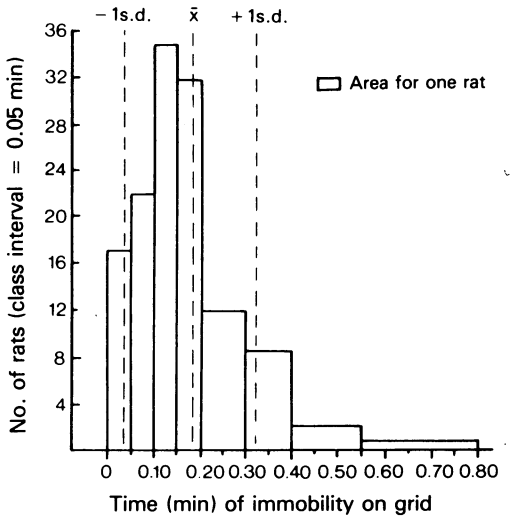

Figure 3 Frequency distribution of the durations of immobility obtained when untreated rats were placed on the vertical grid. The longest time the animal remained in complete immobility during an observation period of 2 min was measured. $n = 157$.

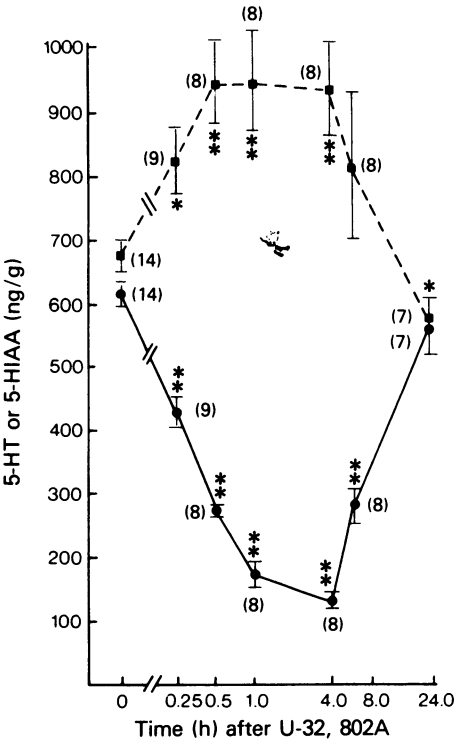

Figure 4 Effect of subcutaneous administration of U-32, 802A 5 mg/kg on the concentrations of 5-hydroxytryptamine (5-HT, ●) and 5-hydroxyindoleacetic acid (5-HIAA, ■) in the pons plus medulla of the rat. Symbols are means and vertical bars represent s.e. mean. Number of estimations indicated in parentheses. * $P < 0.01$, ** $P < 0.001$ vs control (zero time values); Student's t test for non-paired samples.

animals were subjected to the same procedure several times, an appreciable change in their behaviour was observed; the animals did not move away after being placed on the grid, instead they stayed in the same place but moved their head frequently from side to side and seemed completely aware of their surroundings. The movements of the head were interrupted by short periods of immobility which did not show any tendency to increase in length after repetitive testing. The longest immobile episode during an observation period of 2 min was taken as a measurement of normal (control) immobility.

It has been reported that when cataleptic animals are disturbed they regain awareness of their surrounding but relapse after a few seconds into the cataleptic state (Feldberg & Sherwood, 1954; Tedeschi, Tedeschi, Cook, Mattis & Fellows, 1959). This we also observed when cataleptic animals were placed on the grid; the animals started to move away but after a few seconds they stopped and remained completely immobile for a period, the duration of which depended on the intensity of the catalepsy. Observation was continued till the immobility ended, usually with a movement of the head, sometimes with movements of the paws. When tested repeatedly, the absence of head movements was the main difference from control animals. Repetition of the testing procedure did not influence results.

Cataleptic effects of U-32, 802A and of tetrabenazine

In the rat, the administration of U-32, 802A produced a cataleptic state similar to that induced by neuroleptic agents including reserpine. The picture started with a reduction of locomotor activity. Unless disturbed, the rat remained in the same place in the cage with its back hunched and its eyes closed.

The time course of the cataleptic effect produced by U-32, 802A is shown in Figure 1. The latency of onset, duration and intensity of catalepsy were dose-dependent. With a dose of 5 mg/kg the effect started 30 min after the administration of the drug and lasted for 24 h; with the lower doses the cataleptic state was evident only at 1 or 2 h after the injection. With 1 mg/kg the effect had disappeared by 6 h. In mice, the catalepsy produced by U-32, 802A had characteristics similar to those seen in rats. Two hours after

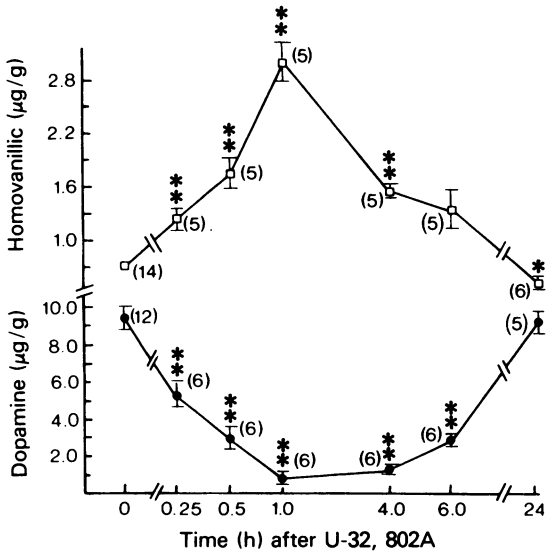

Figure 5 Effect of subcutaneous administration of U-32, 802A 5 mg/kg on the concentrations of dopamine and homovanillic acid in the caudate nucleus of the rat. Symbols are means and vertical bars represent s.e. mean. Number of estimations indicated in parentheses. * $P < 0.05$, ** $P < 0.001$ vs control (zero time values); Student's t test for non-paired samples.

the subcutaneous injection of 2 and 5 mg/kg, the duration of immobility was somewhat longer than in the rat and amounted to means ($\pm$ s.e. mean) of 2.7 ($\pm$0.45) and 5.5 $\pm$ (0.50) min. The values were the same, whether the observations were made during daylight or darkness.

For comparison, the time course of the cataleptic effect in rats of two doses of tetrabenazine is shown in Figure 2. After a dose of 2 mg/kg, the effect was evident 30 min after drug administration and its duration and intensity increased with the dose. The drug was only slightly less effective than U-32, 802A. In contrast, mice required much larger doses of tetrabenazine than of U-32, 802A to show pronounced catalepsy, and 4 to 5 times as much as was needed in rats for a comparable effect. Thus in groups of 9 mice the periods of immobility after 10 mg/kg lasted 0.9 $\pm$ 0.18 min, and after 15 mg/kg 1.85 $\pm$ 0.22 min.

Statistical considerations

The frequency distribution of the periods of immobility obtained when control rats were on the vertical grid is shown in Figure 3. There were higher frequencies among the shorter durations, so the distribution was skew. A chi square (χ^2) test confirmed that the data were not normally distributed ($\chi^2 = 28.4$, d.f. = 7, $P < 0.0005$). The frequency distributions of cataleptic scores from rats treated with U-32, 802A or tetrabenazine also showed most of the frequencies falling within the shorter durations of immobility (U-32, 802A: $\chi^2 = 17.3$, d.f. = 7, $P < 0.001$; tetrabenazine: $\chi^2 = 15.3$, d.f. = 8, $P < 0.025$). A correlation study between the grid test values before and after drug administration showed that in neither instance was there a correlation between pre- and post-drug scores (U-32, 802A: $r = 0.094$, $P = 0.5$, $n = 54$; tetrabenazine: $r = 0.068$, $P = 0.6$, $n = 60$). Thus, the intensity of catalepsy was not related to the duration of the control episodes of immobility (pre-drug scores), and therefore it is not necessary to make any correction to the cataleptic scores or any selection of the experimental animals.

Effect of U-32, 802A on the metabolism of 5-hydroxytryptamine in the brain

The effect of U-32, 802A (5 mg/kg s.c.) on the 5-HT and 5-HIAA content of the rat's combined medulla and pons is shown in Figure 4. There was a large fall in the concentration of 5-HT, which was evident as early as 15 min after the injection, when the amine content was reduced by 30%. The maximal fall was found between 1 and 4 h after the injection (reductions of 72 and 78%). At 6 h the concentration of 5-HT had started to recover (to 54%), and total recovery had occurred at 24 h. The same figure shows that the reduction of 5-HT was accompanied by an elevation in the concentration of 5-HIAA. At 15 min the concentration of the 5-HT metabolite was increased by 22%; at 30 min it was raised by 40% and remained at this level for up to 6 h when its concentration started to decrease towards normal. In the rat midbrain and forebrain, subcutaneous injection of U-32, 802A produced reductions in 5-HT content and accumulation of 5-HIAA of the same magnitude as in the combined medulla and pons. However, when the same dose was given by intraperitoneal injection, the fall in forebrain 5-HT was significantly less (to 225 $\pm$ 19.1 ng/g, $n = 9$) than when the subcutaneous route was used (fall to 125 $\pm$ 12.2 ng/g, $n = 8$).

Mice responded in the same way as rats to subcutaneous injection of U-32, 802A, 5 mg/kg. Within 4 h, the 5-HT concentration had fallen in the forebrain by a mean of 77%, and in the combined midbrain, pons and medulla by 79% ($P < 0.01$). By that time, the concentration of 5-HIAA in the forebrain had risen by 21% and in the other regions by 35% ($P < 0.05$).

Effect of U-32, 802A on the metabolism of dopamine in the striatum

Figure 5 shows the time course of the effect of U-32, 802A injected subcutaneously on the concentration

of dopamine and HVA in the striatum of the rat. The fall in dopamine was somewhat more severe than the fall in 5-HT after the same treatment. The onset was rapid, a 42% reduction being present 15 min after the injection. Between 1 and 4 h the amine content was at its lowest with losses between 90 and 86%. The recovery started at 6 h and the level was normal at 24 h. U-32, 802A also produced a large depletion of dopamine in the striatum of the mouse: 2 h after 2 mg/kg 90% of the dopamine had disappeared. The fall in dopamine was accompanied in both species by a rise in HVA. In rats, the increase reached a peak of 308% at 1 h and rises of 112% and 84% were still seen at 4 and 6 h. In the mouse the rise in HVA amounted to 81% at 2 h.

Effect of U-32, 802A on the concentration of noradrenaline in the brain of the rat

The subcutaneous injection of U-32, 802A induced a large fall in the concentration of NA in the combined hypothalamus-midbrain-pons-medulla. In contrast to the effect of the drug on brain 5-HT and dopamine, there was no change in the concentration of NA 15 min after the injection. At 30 min, however, the NA content had fallen by 34% ($P < 0.01$) and the maximal depletion was obtained at 4 h when the concentration of the amine had been reduced by 87% (fall from control mean of 513 ng/g to 68 ng/g). Two hours later, recovery had started, and at 24 h the NA concentration was reduced by only 29% ($P < 0.05$ versus controls).

Effects of tetrabenazine on the monoamine content of the brain

In rats, 2 h after the subcutaneous administration of tetrabenazine (4 mg/kg), the forebrain showed a reduction of 53% in the concentration of 5-HT and an increase of 64% in that of 5-HIAA (Table 1). The same treatment had a more pronounced effect on the metabolism of dopamine in the striatum. The amine content was reduced by 87%, while the concentration of HVA was raised by 243% (Table 1). However, after tetrabenazine, the NA content of the combined lower brain stem plus hypothalamus fell by only 47% (Table 1).

In mice, a larger dose of tetrabenazine, 10 mg/kg, had to be given to produce changes in amines comparable to those seen in the rat: with that dose the forebrain content of 5-HT fell by 28% and that of 5-HIAA rose by 23%. Striatal dopamine was reduced by 85% while HVA was increased by 127%.

U-32, 802A and body temperature

Some effects of phenothiazines have been related to the hypothermia they produce (Costa, Gessa & Brodie, 1962). It was therefore important to see whether U-32, 802A produced any changes in body temperature which might be considered to have had behavioural or biochemical effects. This was not found to be the case. During the first hour after drug injection, the biochemical changes reached their maximum, and catalepsy, though not maximal, was fully developed. During this period, rectal temperature was unchanged. Subsequently, it fell by no more than 1°C during the time of prolonged sedation, and this slight fall may well have been a consequence, rather than a cause of the cataleptic state.

Discussion

The vertical grid test used in this work was modified from the conventional test, by determining the time during which the animal kept not only its limbs but also its head immobile. The main advantage was that the values so obtained were the same whether the test was carried out once or repeatedly; it also

Table 1 Effects of tetrabenazine on the metabolism of monoamines in the brain of rats

	5-HT (*forebrain*) μg/g	*5-HIAA* (*forebrain*) μg/g	*Dopamine* (*striatum*) μg/g	*HVA* (*striatum*) μg/g	*NA* (*brain stem plus hypothalamus*) μg/g
Control	0.43 ± 0.02 (14)	0.44 ± 0.02 (14)	9.60 ± 0.55 (14)	0.74 ± 0.03 (14)	0.51 ± 0.04
Tetrabenazine (4 mg/kg, s.c.)	0.21 ± 0.02* (6)	0.72 ± 0.03* (5)	1.27 ± 0.15* (7)	2.54 ± 0.17* (10)	0.24 ± 0.02* (6)

Animals were killed 2 h after administration of the drug. Values are expressed as mean ± s.e. mean. Number of estimations in parentheses.

* $P < 0.001$ vs controls; Student's t test for non-paired samples.

avoided the need to use a 'cut-off' when catalepsy was intense, since 10 min was the longest period of total immobility encountered in our experiments. In a recent paper, Costall, Hui & Naylor (1978), replying to criticism by Stanley & Glick (1976), suggest that it is also possible to use the horizontal bar test in such a way that repeated tests give reliable results. However, their procedure is more time-consuming than the modified grid test.

The administration of U-32, 802A was found to induce a dose-dependent cataleptic state, similar in duration to that induced by tetrabenazine. The catalepsy after U-32, 802A was not an unexpected phenomenon since its production is a characteristic effect of butyrophenones and is thought to be due to a blockade of dopamine receptors in the striatum (Maj & Zebrowska, 1966; van Rossum, 1966). However, as already shown by Lahti & Lednicer (1974), U-32, 802A also has a pronounced brain amine depleting activity. Thus, the reduction of brain dopamine content after U-32, 802A is probably also interfering with striatal dopaminergic neurotransmission and this action could also account for the production of catalepsy. In this respect the action of U-32, 802A would be similar to that of tetrabenazine.

The depletion of brain 5-HT we obtained by U-32, 802A in rats is much larger than that seen by Lahti & Lednicer (1974) who measured the brain 5-HT content 4 h after an intraperitoneal injection of 3 mg/kg of the drug. This dose is lower than the one used by us, and that could contribute to the difference in the results. However, the main reason appears to be the finding of a greater efficacy of subcutaneous compared to intraperitoneal administration of U-32, 802A, presumably due to rapid drug metabolism in the liver. The discrepancy between the results is even greater in mice, in which Lahti & Lednicer (1974) obtained severe depletion of catecholamines after intraperitoneal injection of U-32, 802A, but found no significant change in cerebral 5-HT. Perhaps strain differences also contribute to the difference in the results.

The depleting effect of U-32, 802A on brain 5-HT was not restricted to a particular region; probably both cell bodies and nerve endings were affected. Lahti & Lednicer (1974) showed that U-32, 802A induced a long-lasting depletion of catecholamines and a rise in HVA in the brain of the mouse. While observing similar changes in the rat, we found the duration of all drug effects much shorter in this animal. The fact that not only catecholamines but also 5-HT was affected made the similarity to the effects of tetrabenazine very close, and suggests that U-32, 802A interferes with the monoamine storage in a reserpine-like fashion. Decreased motor activity, hunched posture, ptosis and catalepsy are behavioural effects of U-32, 802A reminiscent of reserpine treatment. Lahti & Lednicer (1974) have already pointed to another analogy: they administered U-32, 802A to mice given protriptyline and found that the loss of NA from the heart was unaffected, thus showing that the action of U-32, 802A was probably on the storage vesicle membrane and not on the cell membrane.

The observation that 5-HIAA and HVA remained elevated even when the amines were maximally depleted is similar to the findings with reserpine (Andén *et al.*, 1964) and suggests an intact and even increased synthesis of 5-HT and dopamine after treatment with the butyrophenone. Evidence of an intact capacity for synthesis of 5-HT in the brain of mice treated with U-32, 802A has been obtained by injecting tryptophan (60 mg/kg), after the butyrophenone and measuring the increase in 5-HIAA concentration elicited by the combination (unpublished data).

It appears that U-32, 802A, although chemically related to the butyrophenones, has a spectrum of activity reminiscent of reserpine. This fact is of interest since it might indicate that the receptors on the membrane of the storage granules, the site of action of reserpine-like drugs, and those at the pre- and post-synaptic neuronal membrane, the site of action of butyrophenones and phenothiazines, are not very different. Recently, Saner & Pletscher (1977), studied a new type of benzoquinolizine derivative with activities similar to those of the butyrophenone and phenothiazine neuroleptics and different from the reserpine-like effects of its chemically related congeners. This new benzoquinolizine caused an increase in the turnover of striatal dopamine in doses which either did not alter or decreased only slightly its content of dopamine. They suggested a similarity between receptors for reserpine-like and those for other neuroleptic drugs. This hypothesis is supported by another report stating that chlorpromazine and haloperidol also interfere with the amine storage in adrenal chromaffin granules (Pletscher, 1977). Saner & Pletscher (1977) found that minor variations in the molecule might change the action of a compound from the reserpine type to the neuroleptic type. This statement seems also to be true for a change in the opposite direction as shown by the effects of U-32, 802A. However, the current evidence does not exclude a blocking action of U-32, 802A on the pre- and post-synaptic dopamine receptors.

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