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# Behavioural and biochemical effects of 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride (St 155) on the central nervous system

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1. St 155 (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride) (0.1–2.5 mg/kg subcutaneously) in rats prolonged chloral hydrate sleeping time, inhibited exploratory activity, reduced rotarod performance and pain-induced aggression; in rats and guinea-pigs conditioned avoidance behaviour was inhibited.

2. In most experiments St 155 was 5–7 times more potent than chlorpromazine on a weight basis. In white rats St 155 and chlorpromazine were equi-effective in inhibiting conditioned avoidance behaviour. St 155 was relatively less active than chlorpromazine in lowering body temperature.

3. Chronic treatment with St 155 resulted in a reduction of its hypotensive and sedative effects and caused irritability and spontaneous episodes of severe fighting in white rats.

4. St 155, at doses causing similar conditioned avoidance response inhibition, produced a significant increase in noradrenaline concentration in all brain regions except the striate. Radioisotope studies indicate an increase in noradrenaline storage without significant changes in metabolism.

St 155 (2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride; Catapres, Boehringer Ingelheim) is a potent new hypotensive drug, effective in lowering blood pressure in humans in doses as low as 75 $\mu$g (Michel, Zimmerman, Nassehi & Seraphim, 1966; Bock, Heimsoth, Merguet & Schoenermark, 1966; Davidov, Kakaviatos & Finnerty, 1967; Ng, Phelan, McGregor, Laverty, Taylor & Smirk, 1967). While the mechanism by which St 155 lowers blood pressure has not yet been definitely established, pharmacological studies suggest that its effects are predominantly on the central nervous system (Hoefke & Kobinger, 1966; Nayler, Rosenbaum, McInnes & Lowe, 1966; Kobinger, 1967; Schmitt, Schmitt, Boissier, Guidicelli & Fichelle, 1968). This is supported by the occurrence of sedation as the principal side-effect of St 155 (Michel *et al.*, 1966; Bock *et al.*, 1966; Davidov *et al.*, 1967); other side effects, possibly due to central nervous system actions—for example, depression, disturbed sleep, increase in psychotic behaviour, and irritability—have also been reported (Iisalo & Laurila, 1967; Simpson, Kunz-Bartholini & Watts, 1967; Ng *et al.*, 1967).

St 155 is of interest, therefore, not only as a hypotensive drug with an unusual imidazoline ring structure, but also as a hypotensive drug having its main actions apparently on the central nervous system. For this reason, some central nervous system effects of St 155, on animal behaviour and on the biochemistry of catechol and indole amines, have been investigated. A preliminary report of some of these results has been published (Ng *et al.*, 1967).

## Methods

Male albino rats (200–210 g), black rats (200–300 g) from a purebred strain, albino rats (180–250 g) from a strain with genetic hypertension (Smirk & Hall, 1958; Phelan, 1968) and male and female albino guinea-pigs (500–1,000 g) were used; controls were of the same species, strain, age and sex as the treated animals in all cases. Drugs were given subcutaneously, except where otherwise specified; doses of St 155 and chlorpromazine are given as weights of hydrochloride salt.

Prolongation of choral hydrate sleeping time (Fastier, Speden & Waal, 1957) in response to drug treatment was measured in rats in a heated (29° C) room. In another group of rats temperatures were measured by rectal thermistor, the rats being kept at a room temperature of either 22° or 4° C. Blood pressures were measured in warmed rats lightly anaesthetized with ether using a tail-cuff plethysmographic method (Phelan, 1968).

### *Behavioural tests*

Exploratory behaviour in response to a change of environment was measured in a Y-runway (Steinberg, Rushton & Tinson, 1961); naive rats were observed over a 3 min period for entry of the arms of the runway, rearing up the side of the runway, number of faecal pellets and number of acts of grooming. Spontaneous activity in groups of rats in their home cages was measured at intervals overnight by an isometric recording from a false floor in the cage (Laverty & Meek, unpublished).

Rotarod performance—the ability to stay on a revolving drum (16 cm diameter, 7 or 12 rev/min)—was measured in trained rats over a 1 min period as the number of falls off the drum. Rats were also trained to avoid a 7 sec electric shock to their feet through the grid bars of a cage floor by climbing a pole in response to a 7 sec warning buzzer (Cook & Weidley, 1957). A similar conditioned avoidance response was induced in guinea-pigs using a 7 sec light stimulus to warn of an impending 7 sec shock, with escape to the other compartment of a 2-compartment shuttle cage. In all experiments using learned behavioural responses, one half (usually six–nine rats) of the group of trained animals were given the drug and the other half were given saline as controls. Drugs were given to each sub-group alternatively and in a random sequence of doses. Control observations were pooled because no tendency to change with time was noted.

Fighting behaviour was induced between a pair of mature black rats (300–400 g) by the application of a short, severe electric shock (0.5 sec, 200 V) to the floor grid bars of a small cage in which they were placed (Ulrich & Azrin, 1962; Azrin, Ulrich, Hutchison & Norman, 1964). The response to the shock consisted of the adoption of a characteristic fighting attitude, accompanied by squealing and baring of teeth, and occurred, depending on the pair of rats, in response to 70–90% of the shocks. With both the conditioned avoidance and the pain-induced aggression tests, the

results on each animal or pair using a drug were expressed as a percentage of the frequency of responses observed in the same animal or pair before drug treatment; the means (±S.E.M.) of these percentages were then calculated.

*Biochemical estimations*

Brains were removed from animals after decapitation, dissected into five regions (thalamus-hypothalamus-midbrain, cortex, striate, cerebellum and brain stem), frozen over solid carbon dioxide and stored at −16° C until estimations were made, usually within 48 hr.

Catecholamines and amine metabolites (noradrenaline, dopamine, 3-methoxytyramine and normetanephrine) were separated on a Dowex 50W ion-exchange resin column and estimated fluorimetrically using an iodine oxidation technique (Laverty & Taylor, 1968a). Homovanillic acid was estimated fluorimetrically by a modification of the method of Juorio, Sharman & Trajkov (1966), 5-hydroxytryptamine and total indoles by the method of Ashcroft & Sharman (1962).

The effect of St 155 on the brain metabolism of exogenous $^3$H-dopamine (ring G-$^3$H-3,4-dihydroxyphenylethylamine; Radiochemical Centre, Amersham) was measured by injecting 10 μc $^3$H-dopamine in 20 μl. saline into the left lateral ventricle of rats lightly anaesthetized with ether, using a stereotaxic apparatus. All rats were killed 4 hr after the injection of $^3$H-dopamine; the brains were removed, dissected into regions, and frozen over solid carbon dioxide. The amine, acid and neutral metabolite fractions from each region were separated (Laverty & Taylor, 1968b); a 0.5 ml. sample of each fraction was added to a toluene-ethanol scintillation mixture and the samples counted in a Packard automatic scintillation spectrometer.

## Results

St 155 in moderate and large doses caused a brief initial excitement followed by profound sedation and tranquillization lasting for 4 hr or more; smaller doses caused only sedation. Rats and guinea-pigs showed pronounced piloerection and exophthalmos after St 155.

St 155 prolonged the sleeping time of white rats given chloral hydrate (300 mg/kg intraperitoneally) (Table 1). Between 5 and 10 times as much chlorpromazine by weight was required to produce an effect equivalent to that of St 155. St 155 had a less profound and less prolonged effect on rectal temperature of rats kept at

TABLE 1. *Effect of St 155 and chlorpromazine on the mean duration of anaesthesia (min±S.E.M.) induced by chloral hydrate (300 mg/kg intraperitoneally)*

| | | | |
|---|---|---|---|
| Saline | 1·0 ml./kg | 49·9± 4·0 | (20) |
| St 155 | 0·05 mg/kg | 73·2± 3·6 | (6) |
| | 0·1 | 81·9± 5·5 | (7) |
| | 0·5 | 100·7± 3·0 | (14) |
| Saline | 1·0 ml./kg | 54·6± 6·1 | (16) |
| Chlorpromazine | 0·5 mg/kg | 69·2± 5·1 | (12) |
| | 1·0 | 73·3± 9·3 | (6) |
| | 2·5 | 77·2± 6·3 | (12) |
| | 5·0 | 141·1±12·8 | (7) |

Drugs were given subcutaneously 10 min before the anaesthetic; room temperature was 29° C. The number of rats is given in brackets. Differences between saline and drug-treated groups are significant ($P<0·05$).

22° C and 4° C than equivalent doses of chlorpromazine (Table 2). These large doses of St 155 produced pronounced sedation but failed to produce any signs of catatonia (Wirth, Gösswald, Hörlein, Risse & Kreiskott, 1958); chlorpromazine (20 mg/kg) induced some mild catatonic effects.

*Behavioural studies*

St 155 was approximately 5–7 times more potent than chlorpromazine in inhibiting exploratory movements in a Y-runway (Table 3). At lower doses St 155 seemed to have slightly more effect on rearing activity, whereas chlorpromazine affected the number of entries. St 155 significantly inhibited the number of faecal pellets dropped. However, this is not due solely to an effect on the " emotionality " of the animal (Broadhurst, 1957). A group of eight rats in their home cage and injected with St 155 (0.05 mg/kg) dropped only one faecal pellet in 4 hr whereas the saline-injected group dropped fifty-one and the chlorpromazine group (0.25 mg/kg) dropped thirty-one. It appears that St 155 in rats, as in humans (Ng *et al.*, 1967), causes constipation. Grooming behaviour in the Y-runway is rather erratic, so it is unclear whether St 155 had any definite effect on this behaviour.

TABLE 2. *Effects of St* 155 *and chlorpromazine on rectal temperatures (mean temperature of four rats* ± S.E.M.) *of white rats kept at* 22° C *and* 4° C

| Time after injection (hr) | Saline 1·0 ml./kg | St 155 1 mg/kg | St 155 5 mg/kg | Chlorpromazine 10 mg/kg | Chlorpromazine 20 mg/kg |
|---|---|---|---|---|---|
| *A.* Room temperature at 22° C | | | | | |
| 1 | 37·1±0·2 | 35·4±0·4 | 36·2±0·2 | 34·9±0·1 | 34·1±0·6 |
| 3 | 36·5±0·1 | 35·0±0·4 | 35·2±0·4 | 34·3±0·8 | 33·5±0·8 |
| 5 | 36·7±0·2 | 35·1±0·4 | 35·2±0·5 | 34·3±0·9 | 34·0±0·6 |
| *B.* Room temperature at 4° C | | | | | |
| 1 | 37·6±0·3 | 33·5±0·3 | 32·6±0·2 | 34·8±0·6 | 33·5±0·6 |
| 3 | 37·2±0·1 | 35·3±0·1 | 32·2±0·5 | 33·5±1·2 | 26·1±3·1 |
| 5 | 37·3±0·2 | 36·4±0·3 | 31·8±0·7 | 32·3±2·3 | * |

* Experiment terminated at 3 hr.

TABLE 3. *Effect of St* 155 *and chlorpromazine on the Y-runway activity of naive white and black rats*

| | Dose | No. of Rats | Number of scores (mean ± S.E.M.) Rearings | Entries | Pellets | Grooming |
|---|---|---|---|---|---|---|
| *A.* White rats | | | | | | |
| Saline | 1·0 ml./kg | 32 | 10·8±1·0 | 7·8±0·6 | 3·4±0·3 | 0·7±0·2 |
| St 155 | 0·05 mg/kg | 8 | 5·9±1·5* | 4·9±0·9* | 0·8±0·5‡ | 1·8±0·5† |
| | 0·1 | 8 | 3·1±0·6‡ | 6·0±0·6 | 0·9±0·4‡ | 1·1±0·5 |
| | 0·2 | 6 | 2·7±0·6‡ | 2·7±0·6‡ | 0·2±0·2‡ | 0·5±0·3 |
| | 0·5 | 8 | 0·0±0·0‡ | 0·1±0·1‡ | 0·0±0·0‡ | 0·0±0·0* |
| Chlorpromazine | 0·25 mg/kg | 6 | 8·0±2·6 | 3·8±1·3† | 3·0±0·9 | 0·2±0·2 |
| | 0·5 | 6 | 7·5±2·3 | 3·7±0·9† | 0·8±0·4† | 0·3±0·3 |
| | 1·0 | 6 | 4·8±0·9* | 2·5±0·4‡ | 1·0±0·5 | 0·2±0·2 |
| | 3·0 | 5 | 0·8±0·4‡ | 1·8±0·4‡ | 1·6±0·7 | 0·0±0·0 |
| *B.* Black rats | | | | | | |
| Saline | 1·0 ml./kg | 8 | 17·3±0·8 | 12·9±0·6 | 0·0±0·0 | 0·1±0·1 |
| St 155 | 0·1 mg/kg | 8 | 6·9±1·0‡ | 4·9±0·7‡ | 0·3±0·2 | 0·4±0·2 |
| | 0·5 mg/kg | 8 | 3·0±0·7‡ | 3·9±0·5‡ | 0·0±0·0 | 0·0±0·0 |
| Chlorpromazine | 0·5 mg/kg | 8 | 6·5±1·8‡ | 4·5±1·4‡ | 0·3±0·3 | 0·4±0·2 |
| | 2·5 | 8 | 3·4±0·8‡ | 3·0±0·9‡ | 1·1±0·6 | 0·5±0·3 |

* $P<0·05$; † $P<0·01$; ‡ $P<0·001$.
The animals were tested for 3 min in the runway 1 hr after subcutaneous injection.

Moderate doses of both St 155 and chlorpromazine were required to cause a significant effect on the rotarod performance of rats (Table 4). Again, St 155 was approximately 5 times more potent than chlorpromazine.

Conditioned avoidance behaviour in rats trained to climb a pole to avoid a shock was inhibited by St 155 (Fig. 1). In white rats, St 155 and chlorpromazine were equieffective; however, in black rats there was the usual factor of 5–7 between effective doses of St 155 and chlorpromazine. Conditioned avoidance behaviour in guinea-pigs, using a shuttle cage, was inhibited by St 155, the ED50 being approximately 1.0 mg/kg.

Pain-induced aggression could be induced reliably only in black rats, particularly in those aged 6 months or more. Dose-response curves similar to those found for the effects on conditioned avoidance behaviour were observed for the inhibition by St 155 and chlorpromazine of pain-induced fighting behaviour (Fig. 2).

TABLE 4. *Effect of St 155 and chlorpromazine on the rotarod performance of white rats*

| | | No. of Observations | Slow | Fast |
|---|---|---|---|---|
| Saline | 1·0 ml./kg | 36 | 1·2±0·3 | 4·1±0·6 |
| St 155 | 0·1 mg/kg | 9 | 1·8±0·8 | 5·6±0·8 |
| | 0·5 | 9 | 1·3±0·9 | 6·2±0·9 |
| | 1·0 | 9 | 6·4±1·0* | 11·2±0·1* |
| | 1·0 | 9 | 4·9±1·4* | 9·6±1·4* |
| Saline | 1·0 ml./kg | 27 | 1·8±0·5 | 5·3±0·8 |
| Chlorpromazine | 1·0 mg/kg | 9 | 3·7±1·2 | 5·2±1·6 |
| | 2·0 | 9 | 8·3±0·7* | 11·6±0·9* |

* Difference from saline controls significant $P < 0{\cdot}001$.
The mean scores (± S.E.M.) represent the number of falls per rat in a 1 min period; slow speed was 7 rev/min and fast speed 12 rev/min. The effect was measured 2 hr after subcutaneous injection of the drug.

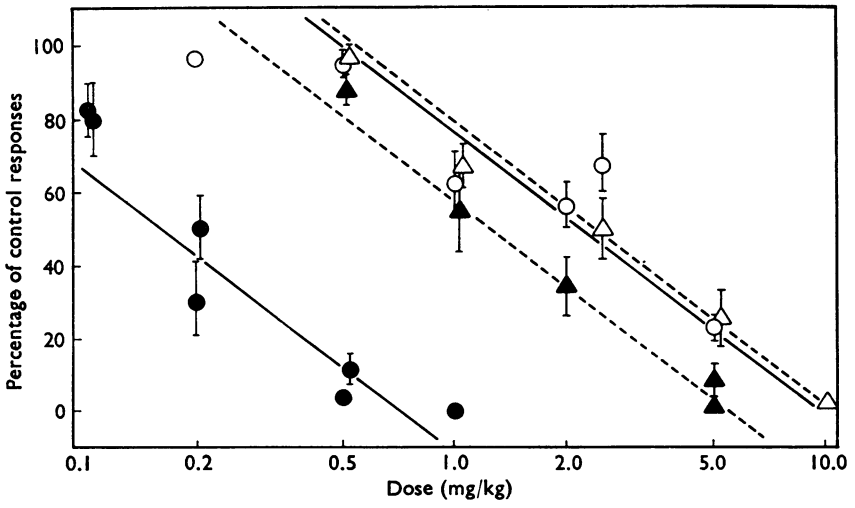


FIG. 1. Effects of St 155 and chlorpromazine on conditioned avoidance behaviour (pole-climbing) in white and black rats. — ● —, Black rats, St 155 treated; --- ▲ ---, black rats, chlorpromazine treated; — ○ —, white rats, St 155 treated; --- △ ---, white rats, chlorpromazine treated. Drugs were given subcutaneously 2 hr before testing; results are expressed as means (±S.E.M., eight rats) of the response of drug-treated animals expressed as a percentage of the response in the same animal without drug on the day before the trial.

*Chronic treatment*

The effect of chronic oral treatment with St 155 on Y-runway behaviour and tail blood pressures was investigated to see whether chronic treatment in rats resulted in the development of tolerance to the actions of the drug.

Two groups each of five normotensive and five genetically-hypertensive rats were used. The drug-treated group was given St 155 (0.2 mg/kg subcutaneously) by injection and then St 155 was added to their glucose-salt drinking fluid (3 mg/l.); the control group was given a saline injection (1 ml./kg subcutaneously) and a similar drinking fluid without St 155. Tail-cuff blood pressures were taken before and during the oral administration (Fig. 3); Y-runway activity was observed 1 hr after the first injection of St 155, and also after a second similar injection, following 7 days of oral treatment. During the second 7 days of treatment the concentration of St 155 in the drinking fluid was increased to 6 mg/l.; the final subcutaneous injection remained the same.

St 155 lowered the blood pressure, particularly in the hypertensive rats (Fig. 3). The maximum falls in blood pressure were not sustained, however, even though the drug intake was increasing, suggesting that tolerance to the hypotensive effects of the drug occurred. In the Y-runway, St 155-injected rats initially showed a depression of exploratory behaviour and defaecation rate; after oral treatment for 7 days, the injection of St 155 had no effect on exploratory behaviour and after oral treatment for 14 days, an increase in exploratory behaviour compared with the controls was actually observed. The defaecation rate was much lower in the treated animals than the controls on all trials. It seems that tolerance to the sedative action but not to the effect of St 155 on faecal pellet release occurred in rats after chronic treatment with St 155.

After 3–6 days of oral treatment or repeated injections with St 155, white rats normally showed slightly reduced nocturnal activity interspersed with violent bouts of activity (Fig. 4). This violent activity was observed during the day and night and consisted of fighting behaviour, involving some or occasionally all members of

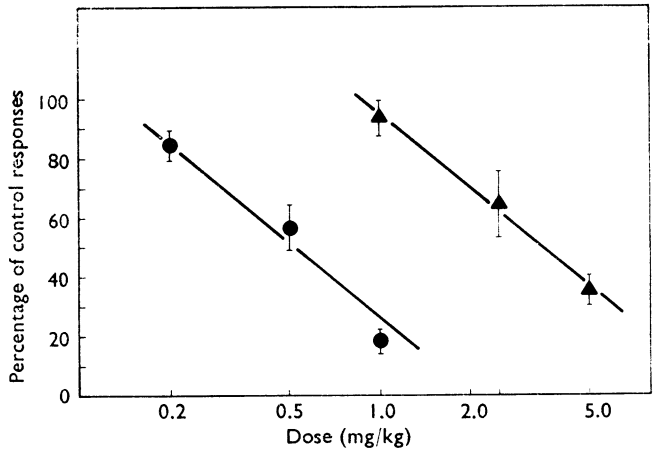


FIG. 2. Inhibition by St 155 and chlorpromazine of pain-induced aggressive behaviour in black rats. Key and treatment as for Fig. 1.

the group. After 14 days on oral administration of the drug, the St 155-treated rats were heavily scarred, indicating the severity and purposefulness of the fighting. Black rats on a similar dose regime, while being slightly sedated, were irritable and difficult to handle but did not show this violent fighting behaviour.

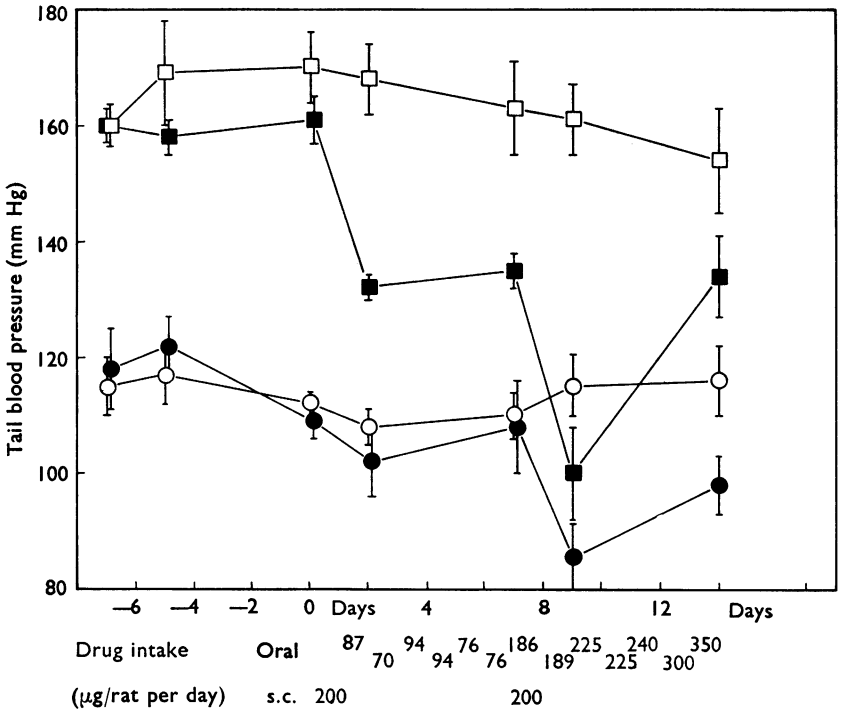


FIG. 3. Effect of St 155 by injection and in the drinking fluid on the tail-cuff blood pressures of genetically hypertensive and normotensive rats. Means (±S.E.M.) are from groups of five rats; the oral drug consumption is the mean consumption per rat per day from ten drug-treated rats. The concentration of St 155 in the drinking fluid was 3 μg/ml. until day 7 and 6 μg/ml. until day 14, —■—, St 155-treated hypertensives; —□—, control hypertensives; — ● —, St 155-treated normotensives; — ○ —, control normotensives.

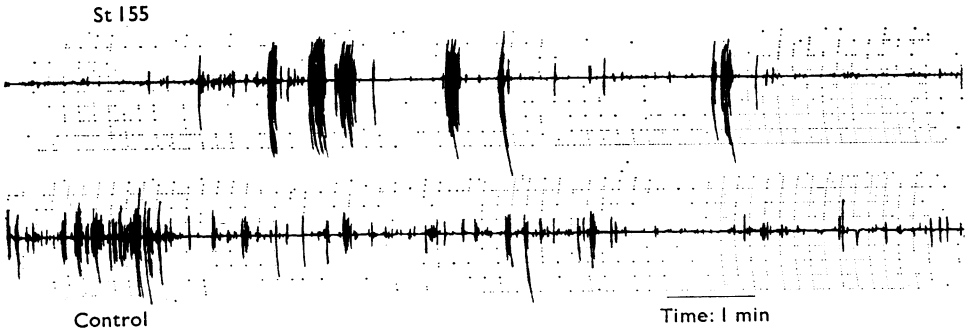


FIG. 4. Isometric recording of activity from a cage of ten white rats treated orally with St 155 and from a control cage of ten white rats. The recording was obtained at 23.00 hr on the ninth day of treatment and indicates the reduced activity of St 155-treated animals interspersed with bouts of violent fighting behaviour, compared with the more continuous activity in the control group. Time marker, 1 min.

*Biochemical studies*

Attempts to estimate the concentration of St 155 in tissues by a variety of fluorimetric techniques were unsuccessful.

No change was found in white rats in the concentration of noradrenaline, normetanephrine, dopamine, 3-methoxytyramine, 5-hydroxytryptamine, total 5-OR indoles or homovanillic acid in whole brain or in various brain regions (thalamic-midbrain, striate, cerebellum, brain stem or cortex) after treatment with St 155 at 0.1 and 0.5 mg/kg for 1, 2, 4 or 8 hr. In all cases, at least four treated and four control animals were examined at each dose and time. At a higher dose level (2.5 mg/kg) in white rats, there was an increase in the noradrenaline concentrations in all brain regions except the striate (Table 5) 2 hr after injection ; some regions also showed an increased noradrenaline concentration 4 hr after injection.

In black rats after a dose of St 155 (0.5 mg/kg) producing a similar behavioural effect (Figs. 1 and 2) there was no change in normetanephrine, dopamine or 3-methoxytyramine in any brain region, but an increase in noradrenaline was observed 2 and 4 hr after injection in all regions except the striate (Table 5).

In guinea-pigs, St 155 (0.5 and 1.0 mg/kg) caused no change in normetanephrine, dopamine, or 3-methoxytyramine in any region, in 5-hydroxytryptamine and total 5-OR indoles in the thalamic-midbrain or in homovanillic acid in the striate. There was a significant increase in noradrenaline concentration in all regions except the striate after 1.0 mg/kg (Table 5).

Chronic treatment of white rats with St 155 (see above ; Fig. 3) had no effect on brain region concentrations of noradrenaline, dopamine or normetanephrine.

Chlorpromazine (5 mg/kg, 2 hr before killing) had no significant effect in white rats on the brain region concentrations of noradrenaline, normetanephrine, dopamine, 3-methoxytyramine, 5-hydroxytryptamine, total 5-OR indoles or homovanillic acid.

TABLE 5. *Effect of St* 155 *on brain regional noradrenaline concentration (means* ± S.E.M.) *in* μg/g) *in white rats, black rats and guinea-pigs*

| Animals, dose and time | No. of Animals | Thalamic-midbrain | Cortex | Cerebellum | Brain stem | Striate |
|---|---|---|---|---|---|---|
| White rats | | | | | | |
| Control | 6 | 0·63±0·01 | 0·22±0·01 | 0·22±0·01 | 0·45±0·02 | 0·31±0·01 |
| St 155 2·5 mg/kg 2 hr | 5 | 0·77±0·01† | 0·29±0·01* | 0·26±0·01* | 0·52±0·01* | 0·31±0·01 |
| 4 hr | 5 | 0·74±0·01† | 0·26±0·01 | 0·22±0·01 | 0·52±0·01* | 0·34−0·02 |
| Black rats | | | | | | |
| Control | 6 | 0·78±0·02 | 0·33±0·01 | 0·26±0·01 | 0·40±0·02 | 0·31±0·02 |
| St 155 0·5 mg/kg 2 hr | 5 | 0·79±0·01 | 0·41±0·01† | 0·33±0·01* | 0·49±0·02* | 0·35±0·03 |
| 4 hr | 5 | 0·84±0·02* | 0·45±0·01‡ | 0·33±0·01* | 0·41±0·01 | 0·35±0·03 |
| Guinea-pigs | | | | | | |
| Control | 4 | 0·28±0·01 | 0·09±0·01 | 0·13±0·01 | 0·32±0·01 | 0·13±0·01 |
| St 155 1·0 mg/kg 2 hr | 4 | 0·34±0·01* | 0·13±0·01† | 0·18±0·01† | 0·41±0·01† | 0·13±0·01 |

Significance of difference from controls: * $P<0·05$; † $P<0·01$; ‡ $P<0·001$.
The doses of drugs are those which inhibit conditioned avoidance behaviour.

*Radioisotope studies*

The separation and estimation of the metabolites of $^{3}$H-dopamine (10 $\mu$c) injected intraventricularly provides a more complete measure of the metabolism of catecholamines in the brain. Metabolites were separated (Laverty & Taylor, 1968a, b) into a basic group from which noradrenaline, normetanephrine, dopamine and 3-methoxytyramine fractions were obtained, and an acid group comprising fractions containing 4-hydroxy-3-methoxy- and 3,4-dihydroxy-mandelic acids, homovanillic acid, and 3,4-dihydroxyphenyl-acetic acid ; the remaining neutral fraction contained the alcohol metabolites. Evidence suggests (Laverty & Taylor, 1968b) that the metabolites in the neutral fractions are derived from labelled amine stored intraneuronally and represent a main metabolic pathway for such intraneuronal amine in the rat. Hence radioisotope studies provide a measure not only of dopamine uptake and conversion to noradrenaline, but also of the turnover of intraneuronal amine as well.

St 155, given to nine rats in two doses of 2.5 mg/kg 2 and 4 hr before killing, caused a significant increase (Table 6) in the mean content of radioactivity present as noradrenaline compared with controls (seven rats) in all brain regions excluding the striate. No other amine, acid, or neutral fraction was significantly affected by St 155 in these regions ; the dopamine contents of these regions were higher in treated animals than in the controls, however. In the striate there was a significant reduction in the content of dopamine and its acid metabolites, 3,4-dihydroxyphenylacetic and homovanillic acids in treated animals compared with controls.

These results suggested that there is an increased storage of noradrenaline in brain tissues, not accompanied by increased metabolism, possibly associated with an increased dopamine uptake and conversion to noradrenaline ; in the striate, there is a reduction in dopamine storage and metabolism.

**Discussion**

In these experiments aspects of the pharmacology of St 155 (Catapres) in rats have been investigated and found to be comparable with those observed in humans.

Measurable behavioural effects occurred at doses smaller or equal to an effective hypotensive dose of St 155 (Table 3, Fig. 3) but many of the observations required much larger doses. The behavioural effects of St 155 were not necessarily related to the hypotensive effects ; a large dose of bethanidine (10 mg/kg subcutaneously) which caused a significant fall in blood pressure (Phelan, personal communication) had no effect on Y-runway, conditioned avoidance response or pain-induced aggressive behaviour in black rats.

The behavioural effects of St 155 were compared with those of chlorpromazine because this drug is the typical member of the sedative tranquillizing group of compounds. Many effects of St 155 were similar to those of chlorpromazine, though St 155 was usually the more potent drug ; differences were observed, however, in the effect of the drugs on temperature regulation and more significantly in the production of irritability and fighting behaviour after chronic treatment with St 155. The hypotensive potency of St 155 is very much greater than that of chlorpromazine. St 155 caused a significant increase in the brain concentration of noradrenaline whereas chlorpromazine had no effect on brain amine metabolism measured fluorimetrically, even after large doses in the rat.

The site of the hypotensive action of St 155 is thought to be in the medulla (Kobinger, 1967) because in cats intra-cisternal injections were more active than intravenous. Experiments in rats, however (Laverty, unpublished), failed to show any increased sensitivity to effects on blood pressure when St 155 was injected into the lateral ventricles of the brain compared with the results of intravenous administration. Nevertheless, there is little evidence for a peripheral hypotensive action (Nayler *et al.*, 1966 ; Rand & Wilson, 1968) and strong suggestive evidence for a central nervous system site of action (Schmitt *et al.*, 1968).

In this respect also, St 155 differs from chlorpromazine as the hypotensive effect of chlorpromazine has a definite peripheral component (Schmitt & Schmitt, 1961). The site of the sedative actions of St 155 is not known ; chlorpromazine is thought to act by depression of the midbrain reticular activating system (Bradley, Wolstencroft, Hösli & Avanzino, 1966).

The development of tolerance to the sedative and hypotensive properties of St 155 has been reported in humans (Ng *et al.*, 1967) ; in the animal experiments chronic treatment caused a corresponding diminution in the response to a test dose of drug in both Y-runway activity and in blood pressure response in rats, suggesting a similar development of toleration of the drug.

The outbursts of vicious fighting behaviour during chronic administration of St 155 is an unusual characteristic of this drug and has not previously been observed with sedative drugs ; amphetamine given repeatedly for more than a month has been reported to induce fighting in rats (Randrup & Munkvad, 1967). The sedation after acute treatment with St 155 contrasts with the lethargic but irritable and pugnacious behaviour during chronic treatment. Tissue concentrations of drug and drug consumption are variable in these chronic experiments and may influence behaviour. It could be that many drugs other than St 155 and amphetamine differ in their acute and chronic behavioural effects ; chronic treatment with chlorpromazine failed to induce any signs of irritability or other unusual behaviour.

A feature of these experiments is the marked differences between strains of rats in their behavioural and biochemical sensitivity to St 155. In many behavioural studies no obvious differences between strains could be detected ; in others, strain differences in behaviour did not affect the response to St 155 : for example, in the Y runway, black rats explore more and defaecate less than white (Table 3 ; Laverty, 1968), but both strains have a similar dose-response curve after St 155. It was in the learned responses that strain differences were most prominent. In the pole-climbing conditioned response behaviour, black rats showed the usual dose ratio of 5–7 between St 155 and chlorpromazine, but in white rats, St 155 and chlorpromazine were equipotent. No obvious explanation of this can be offered. In the pain-induced aggressive behaviour tests, no comparison between strains was possible because white rats could not be induced to fight with any degree of reliability. In contrast, spontaneous fighting behaviour after chronic treatment with St 155 was observed only in white rats, though the black rats were more irritable and difficult to handle while on St 155.

It is interesting that the doses of St 155 which cause a significant change in noradrenaline content in the two strains and in two species are similar to the doses required to inhibit the conditioned avoidance response. The doses involved are much higher than the effective hypotensive dose, so that it is not possible to relate brain biochemical changes to the hypotensive effect ; the parallel between doses

effective on noradrenaline storage and on conditioned avoidance behaviour suggests the possibility of some relationship between behaviour and brain biochemistry. The peak effect of St 155 on brain noradrenaline concentrations occurred at the same time as the peak sedative effect, but more detailed studies on the time course of the effects of St 155 on sedation, irritability, and brain noradrenaline metabolism are required.

The biochemical studies indicate that of the brain monoamines studied, noradrenaline metabolism is the main system affected by St 155. By both fluorimetric and isotopic techniques, high doses of St 155 resulted in an increase in stored noradrenaline in the brain. Drugs which inhibit the enzyme monoamine oxidase also cause an increase in noradrenaline content; however, there was no effect on the content of dopamine, normetanephrine or 5-hydroxytryptamine which should occur if noradrenaline oxidase inhibition was the mode of action of St 155. Metabolism of noradrenaline does not seem to be greatly inhibited by St 155, so that the increased storage may be due to an activation of the dopamine uptake system or the $\beta$-hydroxylation step. At this stage it is not possible to assess how such biochemical changes could relate with the observed physiological and behavioural effects of St 155.

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