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Pharmacological properties of centrally-administered agents which interfere with neurotransmitter function: a comparison with the central depressant effects of ouabain

N. S. DOGGETT* AND P. S. J. SPENCER*

Pharmacology Laboratories, Department of Pharmacy, University of Aston, Birmingham B4 7ET

Summary

1. Centrally administered sodium diethyldithiocarbamate (DDC) produced hypothermia, central nervous depression and potentiation of the antinociceptive effect of morphine. These effects resemble those seen with centrally administered ouabain. Furthermore, the interactions of (+)-amphetamine, desmethylimipramine and nialamide with DDC and ouabain were similar.

2. 6-Hydroxydopamine by the same route also produced central nervous depressant effects including hypothermia, decreased locomotor activity and catalepsy but not ptosis.

3. Both ouabain and chlorpromazine produced similar effects on behaviour and body temperature including selective abolition of a conditioned avoidance response.

4. Although centrally administered tetrabenazine produced ptosis, decreased locomotor activity and catalepsy, it had no significant effect on body temperature. However, the hypothermia produced by peripherally administered reserpine was reversed by centrally administered dibutyryl cyclic 3′,5′-adenosine monophosphate.

5. Centrally administered cocaine and desmethylimipramine produced no depressant effects but an increased excitability and responsiveness were apparent in both cases.

6. Although the observed behavioural depression and hypothermia can occur independently both seem to involve an interference with dopaminergic systems.

Introduction

Previous investigations have shown that, whereas certain doses of ouabain given by intracerebroventricular (icv) injection to mice are excitatory, doses below 0·4 μg produce a dose-dependent depression of central nervous activity (Doggett, Spencer & Turner, 1970; Doggett & Spencer, 1971). This depression is accompanied by a marked increase in dopamine levels and a small non-significant decrease in noradrenaline levels in the brain. It has been postulated that an interference with central transmitter function may be involved in the production of these effects (Doggett & Spencer, 1971).

* Present address: Welsh School of Pharmacy, UWIST, King Edward VII Avenue, Cardiff CF1 3NU

To investigate further the mechanism of action of centrally-administered ouabain, a comparison was made with a number of other drugs given by icv injection. Sodium diethyldithiocarbamate (DDC)—an active metabolite of disulfiram—given by icv injection in rats, increases dopamine levels and decreases noradrenaline levels in the brain (Kleinrok, Zebrowska & Wielosz, 1970). 6-Hydroxydopamine (6-OHDA), however, causes a long lasting depletion of both dopamine and noradrenaline from rat brains (Uretsky & Iversen, 1970). Consequently, comparison of these two agents has been made with icv ouabain.

Studies on the mechanism of amine accumulation by the adrenergic neurone have revealed the existence of two separate amine concentrating mechanisms, one operating as an amine pump at the neurone membrane and the other operating intracellularly at the levels of amine storage granules (Carlsson, 1966; Giachetti & Shore, 1966). The membrane pump is blocked by cocaine, desmethylimipramine, chlorpromazine and ouabain, whereas the granular mechanism is blocked specifically by reserpine and tetrabenazine (Giachetti & Shore, 1966). Therefore, the effects of cocaine, desmethylimipramine, chlorpromazine and tetrabenazine have been compared with ouabain following their icv injection.

Finally, since dibutyryl cyclic 3′,5′-adenosine monophosphate (dbc AMP) antagonizes the effects of icv ouabain, and because icv ouabain produces similar effects to peripherally administered reserpine, the effect of dbc AMP on reserpine-treated mice was also studied.

Methods

Animals

Male albino mice of a TO strain, weighing 16–20 g and male Wistar rats weighing 200–250 g were housed under constant environmental conditions of $21 \pm 1°$ C at a relative humidity of 50–60%. They were allowed free access to drinking water and a conventional 41B cube diet until 2 h before experiment, when both were withdrawn.

Injections

The method used for the icv injection of drugs into mice was a modification of that first described by Haley & McCormick (1957) and subsequently by Brittain & Handley (1967).

In the present experiments, the injection needle was a specially prepared 27 gauge needle, 0·32 cm long. After preliminary investigations, an injection site more rostral and medial than that described by the above workers was decided upon. The needle was inserted perpendicularly through the skull in the region of the bregma, on the mid-line. This site was chosen because it provides adequate penetration of the ventricular system, is easy to find and, in the strains of mice used, the needle can be easily introduced through the skull at this point. Puncture of the sagittal venous sinus which runs beneath the point of entry described by the previous two groups of workers, is avoided.

To check the areas of brain tissue reached after injection, a 5% aqueous solution of pontamine sky blue was injected as described above. The animals were killed by decapitation under chloroform anaesthesia 5 min after injection and the brains dissected out. Inspection of the brain *in situ* after removing the interparietal, parietal and frontal bones revealed no superficial staining of the dorsal surfaces of

the cerebral hemispheres. Coronal sections cut with a freezing microtome revealed penetration of dye throughout the ventricular system.

Icv injections in rats were made using the method of Hayden, Johnson & Maickel (1966) as modified by Sparkes & Spencer (1971).

All drugs given by icv injection were dissolved in 0·9% w/v sterile apyrogenic NaCl solution and administered in a dose volume of 10 μl. The 6-OHDA solution was made up immediately before use and contained 0·5% w/v ascorbic acid. Agents given by peripheral injection were dissolved in 0·9% w/v NaCl solution and administered in a dose volume of 10 ml/kg.

Measurement of body temperature

Oesophageal temperatures were determined as an indication of core temperature of the mice. They were measured according to the method of Brittain & Spencer (1964).

Nociceptive sensitivity in the mouse

The tail-flick method of D'Amour & Smith (1941) was modified so that the light produced by a pre-focussed projector bulb (supply: 6 V DC current at 4·5 A) was directed onto a point 4·5 cm from the base of the tail, the animal being restrained in a glass container with a close fitting lid through which the tail projected. The stimulus caused the mouse to flick its tail away, the end-point being the first escape response. To prevent permanent damage to the animals a cut-off time of 10 s was employed. The stimulus intensity under the above conditions was such that the reaction time of both untreated and control animals lay consistently between 3·5 and 4·5 seconds.

Groups of 10 mice were used for each dose level and the reaction times determined at intervals after administration of the drug. Results are expressed as group mean reaction times, seconds $\pm$ S.E. The significance of inter-group differences was assessed by Student's *t* test, provided not more than 50% of the animals in a group exhibited reaction times greater than the cut-off time.

Measurement of conditioned avoidance behaviour in the rat

The method used was based on that described by Jacobsen & Sonne (1955; 1956), using a commercially available twin-compartment cage (Ugo Basile, Milan, Automatic Reflex Conditioner Cap 4), the animals being trained to escape from a combined audio/visual conditioned stimulus (C.S.) and an electrical unconditioned stimulus (U.S.). Before each experiment, the rat was left undisturbed in the cage for 10–15 minutes. The C.S. was given for a fixed period of 3 seconds. If the rat did not react by running into the other compartment of the cage the electric shock was given for up to 5 s, depending on the presence or absence of an escape response. The stimuli were given at intervals of 20 s, 20 stimuli being given per trial. The relation between conditioned avoidance response (C.A.R.) and unconditioned response (U.R.) was then determined. A training period of seven experiments at intervals of 24 h resulted in 60% of the animals exhibiting C.A.R. for an average of 90% of the stimuli they received, and only these animals were used for further investigations. Subsequently the frequency with which the rat exhibited C.A.R. (% avoid) and the frequency with which the rat failed to exhibit U.R. (% escape failure) was measured in trials of 20 stimuli and compared with similar

results obtained in the control experiment made immediately beforehand in the same rat. Four animals were used at each dose level of every drug tested. An estimation was also made of the length of time during each trial for which the C.S. was administered and this was recorded as the latency.

Results

Effect of icv sodium diethyldithiocarbamate in the mouse

General pharmacological effects

Many authors have described behavioural changes in animals treated with sodium diethyldithiocarbamate (DDC). It has been suggested that these changes are dependent on the depletion of brain noradrenaline or on the increase of brain dopamine (Pfeifer, Galambos & György, 1966; Randrup & Scheel-Krüger, 1966; Scheel-Krüger & Randrup, 1967; Krantz & Seiden, 1968). The behavioural changes described include a reduction of locomotor activity and hypothermia.

Previous observations (Doggett & Spencer, 1971) have shown that mice receiving icv DDC exhibited a dose-dependent hypothermia, which lasted for about 3 h, and was accompanied by catalepsy, ptosis and a reduction in locomotor activity. These effects appeared to be qualitatively similar to those observed after icv ouabain. It was also shown that the effects of icv ouabain could be antagonized by (+)-amphetamine and desmethylimipramine, but not by the MAO inhibitor, nialamide.

The present experiments show that a dose of (+)-amphetamine (2 mg/kg i.p.) which produced no significant hyperthermia alone, immediately reversed the hypo-

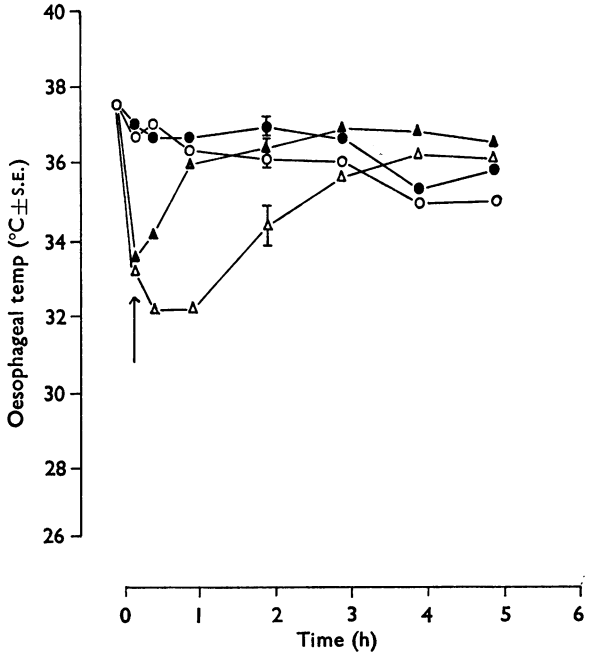

FIG. 1. Effect of (+)-amphetamine on the hypothermia produced by sodium diethyldithiocarbamate (DDC) in the mouse. (○——○) 10 μl saline icv+10 ml/kg saline i.p. at the arrow; (●——●) 10 μl saline icv+2 mg/kg (+)-amphetamine i.p. at the arrow; (△——△) 1 mg DDC icv+10 ml/kg saline i.p. at the arrow; (▲——▲) 1 mg DDC icv+2 mg/kg (+)-amphetamine i.p. at the arrow.

thermia (Fig. 1) and behavioural depression induced by DDC (1 mg icv) if given 15 min after DDC. However, it was found that DDC given 15 min earlier did not sensitize the animals to the hyperthermic effects of a subsequent dose of (+)-amphetamine (10 mg/kg i.p.).

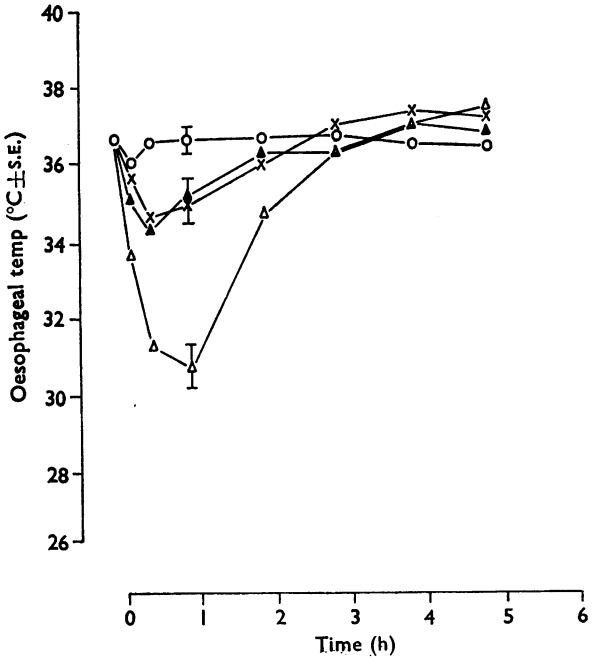

FIG. 2. Effect of desmethylimipramine pretreatment on the hypothermia produced by icv sodium diethyldithiocarbamate (DDC) in the mouse. (○——○) 10 ml/kg saline i.p. 5 min before 10 μl saline icv at time 0; (△——△) 10 ml/kg saline i.p., (×——×) 5 mg/kg desmethylimipramine i.p. or (▲——▲) 10 mg/kg desmethylimipramine i.p. 5 min before 1 mg DDC icv at time 0.

Desmethylimipramine (5 and 10 mg/kg i.p.) pretreatment prevented both the hypothermic (Fig. 2) and behavioural changes of DDC (1 mg icv). This pretreatment also resulted in death from convulsions in mice following icv injection of 1 mg DDC, a dose which did not produce convulsions when administered alone.

In contrast, nialamide (20 mg/kg i.p.) given 2 h previously was unable to antagonize the effects of DDC (1 mg icv), producing instead a small potentiation of the depth of DDC-induced hypothermia, followed by an enhanced rate of recovery (Fig. 3a). This biphasic effect was similar to that seen after icv ouabain (Fig. 3b).

Effect on the antinociceptive activity of morphine

Since it has been shown previously (Calcutt, Doggett & Spencer, 1971) that icv ouabain can potentiate the antinociceptive activity of morphine in mice, it seemed of interest to examine the interaction between DDC and morphine.

Four groups of 10 mice received morphine (2·5 mg/kg s.c.) followed by DDC (20–500 μg icv) or saline (10 μl icv) 15 min later. Three other groups received DDC (100 and 500 μg icv) or saline (10 μl icv) alone. The animals were tested for

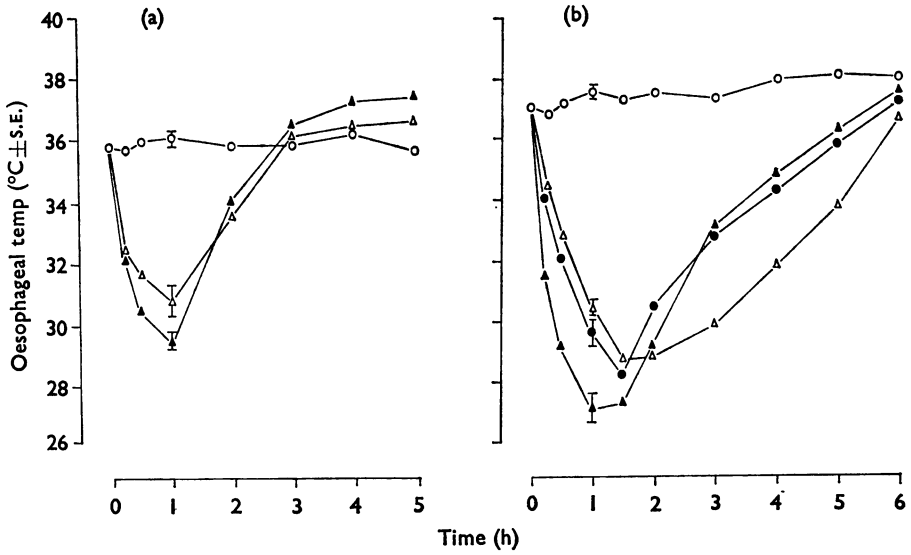

FIG. 3. Effect of nialamide pretreatment on the hypothermia produced by sodium diethyldithiocarbamate and ouabain in the mouse. (a) (○——○) 20 mg/kg nialamide i.p. 2 h before 10 μl saline icv at time 0; (▲——▲) 20 mg/kg nialamide i.p. or (△——△) 10 ml/kg saline i.p. 2 h before 1 mg DDC icv at time 0. (b) (○——○) 10 mg/kg nialamide i.p. 2 h before 10 μl saline icv at time 0; (●——● 10 mg/kg nialamide i.p., (▲——▲ 20 mg/kg nialamide i.p. or (△——△ 10 ml/kg saline i.p. 2 h before 0·3 μg ouabain icv at time 0.

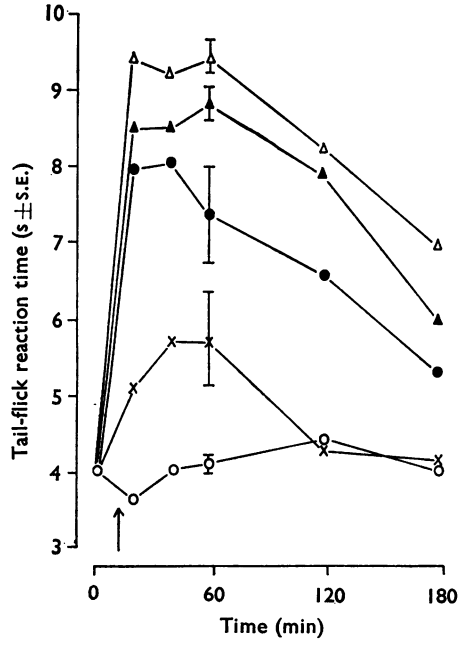

FIG. 4. Effect of icv sodium diethyldithiocarbamate (DDC) on the antinociceptive activity of morphine in the tail-flick test. Mice received (○——○) 10 ml/kg saline s.c. at time 0, 15 min before 500 μg DDC icv at the arrow; 2·5 mg/kg morphine s.c. at time 0, 15 min before (×——×) 10 ml/kg saline icv, (●——●) 20 μg DDC icv, (▲——▲) 100 μg DDC icv, or (△——△) 500 μg DDC icv at the arrow.

nociceptive sensitivity by means of the tail flick test at intervals after injection of morphine or DDC. It was found that icv DDC produced a rapid, marked potentiation of the antinociceptive activity of morphine in doses which, when administered alone, produced no significant increase in tail-flick reaction times (Fig. 4).

Effect of icv 6-hydroxydopamine in the mouse

The injection of 6-OHDA (1–50 μg icv) into four groups of 5 mice was followed by a fall in body temperature of up to 4° C, which lasted for about 2 h, the degree of hypothermia being dose-related (Fig. 5). No ptosis was observed and catalepsy was present only in those animals receiving higher doses (25 and 50 μg icv). All animals showed a decrease in locomotor activity compared with controls, an observation previously made in the rat by Laverty & Taylor (1970).

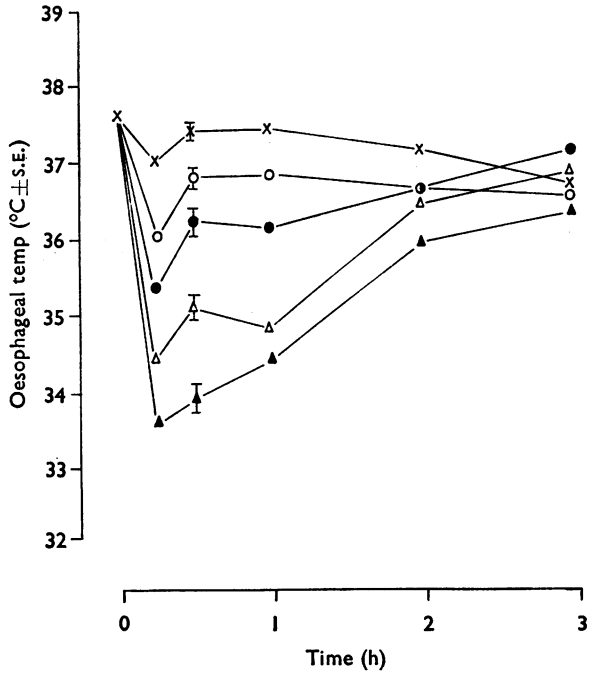

FIG. 5. Effect of icv 6-hydroxydopamine (6-OHDA) on the body temperature of the mouse. (×——×) 10 μl saline; (○——○) 1 μg 6-OHDA; (●——●) 10 μg 6-OHDA; (△——△) 25 μg 6-OHDA; (▲——▲) 50 μg 6-OHDA.

Effect of icv cocaine and desmethylimipramine in the mouse

Cocaine (1–100 μg icv) was injected into four groups of 5 mice. No characteristics of central nervous depression were observed—there was no significant effect on body temperature, no decrease in locomotor activity, no ptosis and no decreased responsiveness to external stimuli. On the contrary, within the dose range investigated the animals showed an increased locomotor activity and excitability and an increased responsiveness to external stimuli which became apparent 5 min after injection and lasted for about 1 hour.

In a further experiment, four groups of 5 mice received desmethylimipramine (1–10 μg icv). Again there was no significant hypothermia or central nervous depression, but an increased excitability and responsiveness similar to that seen after cocaine. No significant effects on locomotor activity were observed.

Effect of icv chlorpromazine and tetrabenazine

General pharmacological effects in the mouse

Chlorpromazine (1–10 μg icv) was injected into groups of 5 mice. A small dose-dependent hypothermia (Fig. 6) accompanied by a decrease in locomotor activity was produced. Catalepsy was present for about 25 min after injection, although no ptosis was seen. The animals exhibited similar but more transient behaviour to that seen after peripheral administration of neuroleptics, and they remained isolated in the cage, not moving until touched.

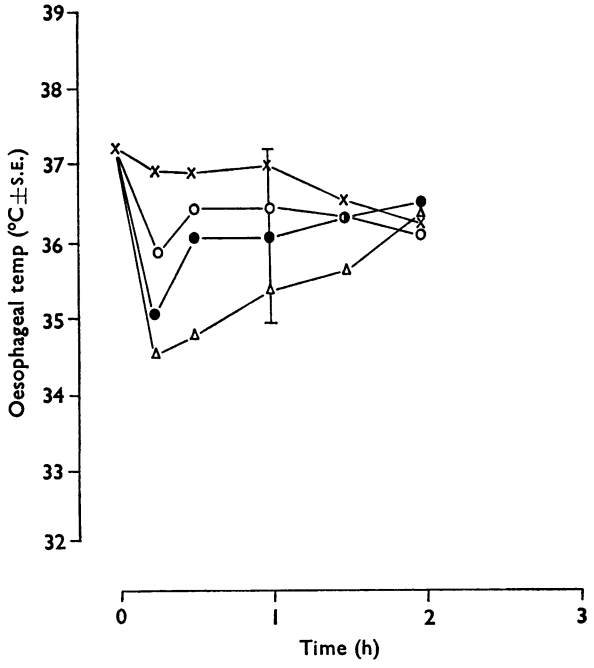

FIG. 6. Effect of icv chlorpromazine (CPZ) on the body temperature of the mouse. (×——×) 10 μl saline; (○——○ 1 μg CPZ; (●——●) 5 μg CPZ; (△——△) 10 μg CPZ.

In an experiment to investigate the pharmacological properties of a centrally acting reserpine-like drug after icv injection, tetrabenazine (1–50 μg icv) was given at five dose levels to groups of 5 mice. It produced no significant effect on body temperature. Within 1 min of injection marked ptosis appeared together with a decrease in locomotor activity, and catalepsy developed after 5 minutes. Ptosis had disappeared within 90 min and catalepsy within 20 min of injection, the animals rapidly becoming indistinguishable from controls. Except for the lack of hypothermia, icv tetrabenazine produced similar effects to those produced by peripherally-administered reserpine, although they had a much shorter time course.

Effect of icv chlorpromazine and icv ouabain on conditioned avoidance behaviour in the rat

To characterize further the effects of icv ouabain, its ability to modify conditioned avoidance behaviour in the rat was investigated. Ouabain (0·3 μg icv) produced no effect on either conditioned avoidance response (C.A.R.) or unconditioned response

(U.R.) for up to 20 h after injection. Higher doses (0·5–1 μg icv) abolished the C.A.R. from 10 min until more than 2 h after injection. The U.R. was unaffected. All animals recovered fully within 20 h (Table 1).

TABLE 1. *Effect of ouabain given by icv injection on conditioned avoidance behaviour in groups of 4 rats. Control received saline (10 μl icv) 10 min before trial. 1 latency unit=0·08 s*

Dose (μg)	Parameter measured	Control	Mean responses (%±S.E.) 10 min after inj.	30 min after inj.	2 h after inj.	20 h after inj.
0·3	% Avoid	100	95	100	98± 2	100
	% Escape failure	0	0	0	0	0
	Latency	268±38	276±15	251±11	260±23	325±12
0·5	% Avoid	87± 6	36± 9	33±19	40± 5	100
	% Escape failure	0	0	5± 5	0	0
	Latency	438±32	805±29	794±170	638±54	301±14
1·0	% Avoid	85± 4	27±12	22± 9	20±0·3	91± 6
	% Escape failure	0	5± 2	2± 2	13± 8	0
	Latency	446±43	932±124	724±38	1,064±227	409±39

In view of the marked similarity of icv ouabain to the effect of peripherally-administered neuroleptics on conditioned avoidance behaviour (Herz, 1960), the activity of centrally-administered chlorpromazine on the conditioned reflex was next studied. Groups of 4 rats received chlorpromazine (50 μg icv) and were tested 10 min after injection. This dose produced a selective abolition of C.A.R. with no effect on U.R. (Table 2). A decrease in locomotor activity was apparent within

TABLE 2. *Effect of chlorpromazine given by icv injection on conditioned avoidance behaviour in groups of 4 rats. Controls received saline (10 μl icv) 10 min before trial. 1 latency unit=0·08 s*

Dose (μg)	Parameter measured	Control	Mean responses (%±S.E.) 10 min after inj.	30 min after inj.
50	% Avoid	95± 3	0	93± 3
	% Escape failure	0	0	0
	Latency	404±20	1,060±180	387±53

2 min of injection and catalepsy developed within 8 minutes; as in the mouse, no ptosis was observed. C.A.R. returned to control levels within 30 min of injection. Thus icv ouabain and icv chlorpromazine are both capable of selectively abolishing C.A.R. at dose levels which have little effect on U.R.

Effect of icv dbc AMP on reserpine-induced hypothermia and behavioural depression in the mouse

Earlier experiments (Doggett & Spencer, 1971) showed that icv dbc AMP can antagonize the hypothermia and behavioural depressant effects produced by icv ouabain. Since icv ouabain produces behavioural effects similar to those of peripherally-administered reserpine, and because Abdulla & Hamadah (1970) have reported that reserpine ptosis can be reversed by dbc AMP, the interaction between icv dbc AMP and peripherally-administered reserpine was examined.

Two groups of 8 mice received reserpine (2 mg/kg i.p.) 20 h before the administration of dbc AMP (25 μg icv) or saline (10 μl icv). The body temperature of the

mice fell to about 25° C at 20 h after reserpine. The group receiving icv dbc AMP showed initially a greater rate of recovery of body temperature towards normal levels compared with the group receiving icv saline. This became apparent 1 h after icv injection and reached a maximum after 2 h (Fig. 7). There was, however,

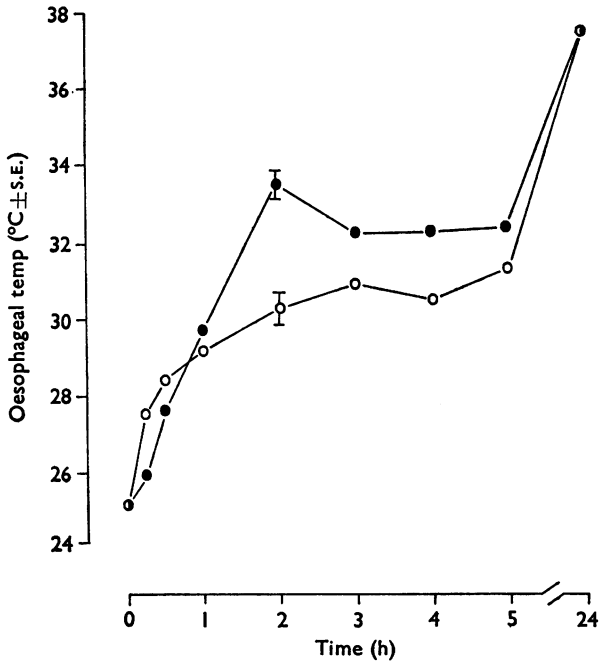

FIG. 7. Effect of icv dibutyryl cyclic 3′,5′-adenosine monophosphate (dbc AMP) on reserpine-induced hypothermia in the mouse. Animals received 2 mg/kg reserpine i.p. 20 h before (○——○) 10 μl saline icv or (●——●) 25 μg dbc AMP icv at time 0.

no observable reversal of the reserpine-induced ptosis produced by icv dbc AMP, nor any marked reversal of the associated behavioural depression.

Discussion

There is an overall similarity in the pharmacological effects of icv ouabain and icv DDC. The hypothermia produced by centrally-administered sodium diethyldithiocarbamate (DDC) is of a qualitatively similar nature to, and of approximately the same duration as, that reported after its peripheral administration (Barnett & Taber, 1968). After icv injection, however, DDC acted in a dose approximately 600 times less than the peripheral doses used by the earlier workers.

The hypothermic effects of both icv ouabain and icv DDC could be antagonized by (+)-amphetamine and desmethylimipramine, whereas nialamide has a biphasic effect against both ouabain and DDC. Unlike ouabain, DDC fails to sensitize the animals to the hyperthermic effect of (+)-amphetamine. Ouabain does sensitize them to both the behavioural and hyperthermic effects of (+)-amphetamine (Doggett, 1971), and this probably reflects the ability of ouabain to block amine uptake in nerve terminals. In addition Maj & Przegaliński (1967) demonstrated that disulfiram could block amphetamine-induced hyperactivity in mice, thus

supporting an antagonism of, but not a sensitization to, the effects of amphetamine by DDC.

The depletion of noradrenaline, as well as the elevation of dopamine, levels in the brain could play a part in the production of the pharmacological effects following the administration of DDC. Kleinrok *et al.* (1970) showed that, following its icv administration in rats, a marked fall in body temperature and motor activity occurred, co-incident with maximum decreases of brain noradrenaline and concomitant increases of brain dopamine. In view of this, desmethylimipramine may antagonize the hypothermia and behavioural depressant effects of icv DDC by elevating the concentration of noradrenaline at the receptor sites after injection of DDC.

Barnett & Taber (1968) found that pretreatment with a monoamine oxidase inhibitor did not significantly affect the hypothermia produced by peripherally-administered DDC in mice. The biphasic effect of monoamine oxidase inhibition seen in the present experiments could be explained by an alteration in both noradrenaline and dopamine levels intracellularly within the brain, since both of these may be elevated after nialamide pretreatment.

A further similarity between icv ouabain and icv DDC is their effect on the antinociceptive activity of morphine. In an earlier paper (Calcutt *et al.*, 1971) it was shown that icv ouabain produced an immediate potentiation of morphine. Watanabe, Matsui & Iwata (1969) also showed potentiation of the antinociceptive activity of morphine by DDC given peripherally to rats, and this has been confirmed in mice, again following peripheral administration of DDC (Maj, Sowińska, Baran & Durek, 1971 ; Zebrowska-Lupina, Kleinrok & Smolarz, 1971). In contrast, Vedernikov & Afrikanov (1969) found that disulfiram, which has a similar inhibitory effect on dopamine-β-hydroxylase (Carlsson, Lindqvist, Fuxe & Hökfelt, 1966), given peripherally, inhibited the antinociceptive activity of morphine in rats.

The possibility of an effect on the metabolism of morphine cannot be excluded in the case of DDC, in view of its known ability to interfere with enzymes other than dopamine-β-hydroxylase (Thorn & Ludwig, 1962). However, an interference with the metabolism of morphine seems unlikely after icv injection of DDC when one considers the route of administration of DDC, the dose of DDC used and the rapidity of onset of potentiation. As suggested in our earlier work, an elevation in brain dopamine levels seems likely to play a part in the interactions of both icv ouabain and icv DDC with morphine.

Although icv 6-OHDA results in a depletion of noradrenaline and dopamine from rat brain (Ungerstedt, 1968 ; Uretsky & Iversen, 1969 ; 1970), it has been suggested (Simmonds & Uretsky, 1970) that 6-OHDA may also release endogenous dopamine in an active form in the brain which causes the observed hypothermia. If this is so, it supports the suggestion that the hypothermia produced by icv ouabain also primarily involves a dopaminergic mechanism, particularly since depressant doses of icv ouabain produce an elevation of whole-brain dopamine levels with no effect on noradrenaline (Doggett & Spencer, 1971). Ouabain inhibits the membrane pump, and this appears to include an interference with dopamine as well as noradrenaline uptake (Goldstein, Ohi & Backstrom, 1970). Desmethylimipramine also blocks the uptake of noradrenaline, but with no effect on dopamine (Carlsson, Fuxe, Hamberger & Lindqvist, 1966 ; Ross & Renyi, 1967 ; Fuxe & Ungerstedt, 1968) ; desmethylimipramine, however, does not produce depressant effects. Fur-

thermore, cocaine, which blocks both noradrenaline and dopamine uptake in mouse brain slices (Ross & Renyi, 1967) produces behavioural excitation.

Thus the depressant effects of icv ouabain appear not only to involve a dopaminergic mechanism but may more specifically contain a component of dopamine receptor blockade. In addition Carlsson & Lindqvist (1967) have emphasized the importance of a functional dopaminergic system for maintaining normal exploratory and locomotor activity. A blockade of dopamine receptors may also explain the effect of icv ouabain on conditioned behaviour, since neuroleptics, including chlorpromazine, which also blocks the conditioned avoidance response after icv injection, can block dopamine receptors in the brain (Carlsson & Lindqvist, 1963; Rossum, 1966; Pletscher & Da Prada, 1967). Experiments currently being carried out in these laboratories also tend to suggest that a blockade of dopamine receptors plays a part in the depressant effect of icv ouabain.

Thus the behavioural depression and hypothermia produced by centrally-administered ouabain, DDC and 6-OHDA resemble those produced by peripherally-administered reserpine. Furthermore, centrally-administered ouabain, DDC, 6-OHDA and chlorpromazine all produced comparable effects (icv tetrabenazine also produced behavioural depression, but had little effect on body temperature). Although the variation in effect on endogenous brain amine levels and on amine uptake serves to re-emphasize the need for caution when interpreting the mechanisms by which each of these drugs interferes with behaviour and thermoregulation, an involvement of dopaminergic systems seems to be a common factor. Further examination of the ouabain-induced effects is currently taking place and it is hoped that a specific causative mechanism will emerge.

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REFERENCES

ABDULLA, Y. H. & HAMADAH, K. (1970). 3',5'-cyclic adenosine monophosphate in depression and mania. *Lancet*, **1**, 378–381.

BARNETT, A. & TABER, R. I. (1968). The effects of diethyldithiocarbamate and L-DOPA on body temperature in mice. *J. Pharm. Pharmac.*, **20**, 600–604.

BRITTAIN, R. T. & HANDLEY, S. L. (1967). Temperature changes produced by the injection of catecholamines and 5-hydroxytryptamine into the cerebral ventricles of the conscious mouse. *J. Physiol., Lond.*, **192**, 805–813.

BRITTAIN, R. T. & SPENCER, P. S. J. (1964). Measurement of body temperature in conscious small laboratory animals by means of an oesophageal thermocouple. *J. Pharm. Pharmac.*, **16**, 497–499.

CALCUTT, C. R., DOGGETT, N. S. & SPENCER, P. S. J. (1971). Modification of the anti-nociceptive activity of morphine by centrally-administered ouabain and dopamine. *Psychopharmacologia*, **21**, 111–117.

CARLSSON, A. (1966). Modification of sympathetic function. Pharmacological depletion of catecholamine stores. *Pharmac. Rev.*, **18**, 541–549.

CARLSSON, A. & LINDQVIST, M. (1963). Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. *Acta pharmac. tox.*, **20**, 140–144.

CARLSSON, A. & LINDQVIST, M. (1967). Metatyrosine as a tool for selective protection of catecholamine stores against reserpine. *Eur. J. Pharmac.*, **2**, 187–192.

CARLSSON, A., FUXE, K., HAMBERGER, B. & LINDQVIST, M. (1966). Biochemical and histochemical studies on the effects of imipramine-like drugs and (+)-amphetamine on central and peripheral catecholamine neurons. *Acta physiol. scand.*, **67**, 481–497.

CARLSSON, A., LINDQVIST, M., FUXE, K. & HÖKFELT, T. (1966). Histochemical and biochemical effects of diethyldithiocarbamate on tissue catecholamines. *J. Pharm. Pharmac.*, **18**, 60–62.

D'AMOUR, F. E. & SMITH, D. L. (1941). A method for detecting loss of pain sensation. *J. Pharmac. exp. Ther.*, **72**, 74–79.

DOGGETT, N. S. (1971). Neuropharmacological effects of certain agents which interfere with cell metabolism. Ph.D. thesis, University of Aston.

DOGGETT, N. S. & SPENCER, P. S. J. (1971). Pharmacological properties of centrally administered ouabain and their modification by other drugs. *Br. J. Pharmac.*, **42**, 242–253.

DOGGETT, N. S., SPENCER, P. S. J. & TURNER, T. A. R. (1970). The pharmacological effects of ouabain administered intracerebrally to conscious mice. *Br. J. Pharmac.*, **40**, 138–139P.

FUXE, K. & UNGERSTEDT, U. (1968). Histochemical studies on the effect of (+)-amphetamine, drugs of the imipramine group and tryptamine on central catecholamine and 5-hydroxytryptamine neurons after intraventricular injection of catecholamines and 5-hydroxytryptamine. *Eur. J. Pharmac.*, **4**, 135–144.

GIACHETTI, A. & SHORE, P. S. (1966). Studies *in vitro* of amine uptake mechanisms in heart. *Biochem. Pharmac.*, **15**, 607–614.

GOLDSTEIN, M., OHI, Y. & BACKSTROM, T. (1970). The effect of ouabain on catecholamine biosynthesis in rat brain cortex slices. *J. Pharmac. exp. Ther.*, **174**, 77–82.

HALEY, T. J. & MCCORMICK, W. G. (1957). Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *Br. J. Pharmac. Chemother.*, **12**, 12–15.

HAYDEN, J. F., JOHNSON, L. R. & MAICKEL, R. P. (1966). Construction and implantation of a permanent cannula for making injections into the lateral ventricle of the rat brain. *Life Sci.*, **5**, 1509–1515.

HERZ, A. (1960). Drugs and the conditioned avoidance response. *Int. rev. Neurobiol.*, **2**, 229–277.

JACOBSEN, E. & SONNE, E. (1955). The effect of benzilic acid diethylaminoethylester, HCl (Benactyzine) on stress-induced behaviour in the rat. *Acta pharmac tox.*, **11**, 135–147.

JACOBSEN, E. & SONNE, E. (1956). The effect of benactyzine on the conditioned responses in the rat. *Acta pharmac. tox.*, **12**, 310–320.

KLEINROK, Z., ZEBROWSKA, I. & WIELOSZ, M. (1970). Some central effects of diethyldithiocarbamate administered intraventricularly in rats. *Int. J. Neuropharmac.*, **9**, 451–455.

KRANTZ, K. D. & SEIDEN, L. S. (1968). Effects of diethyldithiocarbamate on the conditioned avoidance response of the rat. *J. Pharm. Pharmac.*, **20**, 166–167.

LAVERTY, R. & TAYLOR, K. M. (1970). Effects of intraventricular 2,4,5-trihydroxyphenylethylamine (6-hydroxydopamine) on rat behaviour and brain catecholamine metabolism. *Br. J. Pharmac.*, **40**, 836–846.

MAJ, J. & PRZEGALIŃSKI, E. (1967). Disulfiram and some effects of amphetamine in mice and rats. *J. Pharm. Pharmac.*, **19**, 341–343.

MAJ, J., SOWIŃSKA, H., BARAN, L. & DUREK, B. (1971). The influence of agents depressing the catecholamine levels on the action of narcotic analgesics. *Diss. pharm. pharmac.*, **23**, 9–23.

PFEIFER, A. K., GALAMBOS, E. & GYÖRGY, L. (1966). Some central nervous properties of diethyldithiocarbamate. *J. Pharm. Pharmac.*, **18**, 254.

PLETSCHER, A. & DA PRADA, M. (1967). Mechanism of action of neuroleptics. In: *Neuropsychopharmacology*. Proceedings of the fifth international congress of the collegium internationale neuro-psychopharmacologicum, Int. Congress Series, No. 129, pp. 304–311. Amsterdam: Excerpta Medica Foundation.

RANDRUP, A. & SCHEEL-KRÜGER, J. (1966). Diethyldithiocarbamate and amphetamine stereotype behaviour. *J. Pharm. Pharmac.*, **18**, 752.

ROSS, S. B. & RENYI, A. L. (1967). Inhibition of the uptake of tritiated catecholamines by antidepressant and related agents. *Eur. J. Pharmac.*, **2**, 181–186.

ROSSUM, J. M. VAN (1966). The significance of dopamine-receptor blockade for the mechanism of action of neuroleptic drugs. *Archs Int. Pharmacodyn. Thér.*, **160**, 492–493.

SCHEEL-KRÜGER, J. & RANDRUP, A. (1967). Stereotype hyperactivity behaviour produced by dopamine in the absence of noradrenaline. *Life Sci.*, **6**, 1389–1398.

SIMMONDS, M. A. & URETSKY, N. J. (1970). Acute hypothermic response of the rat to the intraventricular injection of 6-hydroxydopamine. *Br. J. Pharmac.*, **40**, 564–565P.

SPARKES, C. G. & SPENCER, P. S. J. (1971). Antinociceptive activity of morphine after injection of biogenic amines in the cerebral ventricles of the conscious rat. *Br. J. Pharmac.*, **42**, 230–241.

THORN, G. D. & LUDWIG, R. A. (1962). *The dithiocarbamates and related compounds*. Amsterdam: Elsevier.

UNGERSTEDT, U. (1968). 6-Hydroxydopamine induced degeneration of central monoamine neurones. *Eur. J. Pharmac.*, **5**, 107–110.

URETSKY, N. J. & IVERSEN, L. L. (1969). Effect of 6-hydroxydopamine on noradrenaline-containing neurones in the rat brain. *Nature, Lond.*, **221**, 557–559.

URETSKY, N. J. & IVERSEN, L. L. (1970). Effects of 6-hydroxydopamine on catecholamine containing neurones in the rat brain. *J. Neurochem.*, **17**, 269–278.

VEDERNIKOV, Y. P. & AFRIKANOV, I. I. (1969). On the role of a central adrenergic mechanism in morphine analgesic action. *J. Pharm. Pharmac.*, **21**, 845–847.

WATANABE, K., MATSUI, Y. & IWATA, H. (1969). Enhancement of the analgesic effect of morphine by sodium diethyldithiocarbamate in rats. *Experientia*, **25**, 950–951.

ZEBROWSKA-LUPINA, I., KLEINROK, Z. & SMOLARZ, J. (1971). The effect of sodium diethyldithiocarbamate on the action of analgesics in albino mice. *Diss. pharm. pharmac.*, **23**, 33–39.

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