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Hypothermic and toxic actions of 2-butyne-1, 4-diol and other related diols in the rat

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The effects of 2-butyne-1,4-diol (BYDL) and related congeners on the body temperature of the rat have been determined. BYDL, at doses above 0·4 mmol kg^{-1} intraperitoneally, produced a significant fall in body temperature which was dose dependent. 2-butene-1,4-diol (BEDL) had no hypothermic action, and butane-1,4-diol (BDL) produced only a moderate fall in temperature (1·9°) which correlated with the time course of the hypnotic effect of the drug. γ-Butyrolactone (GBL), γ-hydroxybutyric acid (GHB) and pentobarbitone at hypnotic doses had similar hypothermic actions which correlated with the period of hypnosis. The hypothermia produced by BYDL could not be prevented by pretreatment with scopolamine or by maintaining the rats at high environmental temperature (32°). BYDL was far more toxic than the other diols examined (LD50 = 0·609–0·635 mmol kg^{-1} compared to BEDL: 3·71–3·74 mmol kg^{-1}; and BDL: 11·87–11·90 mmol kg^{-1}). Pretreatment of rats with pyrazole, an inhibitor of liver alcohol dehydrogenase, prevented the toxic and hypothermic actions of BYDL. From earlier studies with pyrazole and BDL it was concluded that BYDL itself was not active but that it was possibly converted *in vivo* by liver alcohol dehydrogenase to a toxic metabolite.

2-Butyne-1,4-diol (BYDL) has many industrial applications (Flickinger & Adolphi, 1963; Leupold, Sponsel & Hillmer, 1966; Cope, 1967), and is particularly widely used as a brightening agent in electrolytic processes (Froment, Maurin & Thevenin, 1968). However, little is known of its pharmacological or toxicological properties, although its behavioural effects relative to butane-1, 4-diol (BDL) and γ-hydroxybutyric acid (GHB) have been reported briefly (Sprince, Josephs & Wilpizeski, 1966).

The present study arose from an interest in the hypnotic and hypothermic actions of BDL and its metabolite GHB. Earlier work in this, and other laboratories, had indicated that BDL itself was inactive and was metabolized to GHB in the liver (Kerkut & Taberner, 1971; Bessman & McCabe, 1972; Maxwell & Roth, 1972; Taberner, Rick & Kerkut, 1972). We have examined the unsaturated analogues of BDL, namely 2-butene-1,4-diol (BEDL) and BYDL in order to determine if they were themselves pharmacologically active products and, if so, whether an increase in potency could be observed with increasing unsaturation of the molecule.

MATERIALS AND METHODS

The rats were adult Wistar albinos of either sex, 320–360 g. All the drugs were made up in 0·9 % (w/v) saline and injected intraperitoneally. GHB was obtained from Ralph Emanuel Ltd., Wembley, England. Sodium pentobarbitone was a gift from Abbott Laboratories, Queensborough, Kent. All other drugs and reagents were

from BDH, Poole. BDL was redistilled twice at 760 mm before use; the butyne-1,4-diol was redistilled at 15 mm then recrystallized twice from ethyl acetate.

Rat body temperatures were measured by inserting a thermistor probe 4 cm into the rectum. The probe was connected to a Wheatstone bridge circuit for which a calibration curve of temperature against resistance had been prepared using a mercury in glass thermometer ($\pm 0{\cdot}02°$).

Values for the LD50 were calculated from the mortality rates in 5 groups of 6 rats using the method of Weil (1952). The partial purification and assays of alcohol dehydrogenase activity from rat liver were according to Bonnichsen & Brink (1955).

Statistical analyses were performed where appropriate using Student's *t*-test for measuring the significance of the difference between the means of independent groups.

RESULTS

Toxicity of 4-carbon diols. Rats were injected with graded doses of BDL, BEDL or BYDL and the LD50 values calculated from the mortality rates (see Table 1).

Table 1. *Toxicity of 4-carbon diols in the rat.* Groups of 6 rats were injected at the dose levels shown (mmol kg^{-1}, i.p.), and the mortality determined after 18 h. Survivors were observed for a further 24 h. Pyrazole was given (i.p.) 10 min before the diol and the mortality determined at 36 h. LD50 values were calculated by the method of Weil (1952).

Butanediol		Butenediol		Butynediol	
Dose	Number of animals dead	Dose	Number of animals dead	Dose	Number of animals dead
8·90	0/6	3·20	0/6	0·558	1/6
10·78	2/6	3·52	1/6	0·614	2/6
12·94	4/6	3·87	5/6	0·675	5/6
15·53	5/6	4·26	5/6	0·743	6/6
17·80	6/6	4·68	6/6	0·817	6/6
17·80	0/6	6·40	0/6	1·116	0/6
+ pyrazole 2·9		+ pyrazole 2·9		+ pyrazole 2·9	
LD50 (95% confidence limits)					
11·87 — 11·90		3·71 — 3·74		0·609 — 0·635	

The results show that increasing unsaturation of the diol produces a corresponding increase in lethality of the diol. In terms of LD50 values, BEDL was 3·2 times more potent than BDL and BYDL was 6 times more potent than BEDL. In each case, however, a dose of 2·9 mmol kg^{-1} (i.p.) pyrazole 10 min before the diol was able to prevent any symptoms of toxicity from developing and also caused rats given LD100 doses of any of the diols to survive indefinitely.

BDL, at sub-lethal doses (<10 mmol kg^{-1}), produced a characteristic hypnotic state, with loss of the righting reflex and maintained muscle tone, after a latency of about 20 min after the injection. Increasing the dose produced an increase in the depth of hypnosis together with a marked bradycardia, analgesia and laboured respiration. Death appeared to be due to respiratory failure.

BEDL produced few behavioural changes at doses below 3·5 mmol kg^{-1}. Higher

doses produced sedation and a loss of spontaneous activity 30–40 min after injection and lasting 2–3 h at which time most rats entered into tonic convulsions and died within 40 min.

BYDL produced very distinctive behavioural effects after the injection of doses of 0·20 mmol kg^{-1} or above. After 15 min a marked increase in parasympathetic activity was apparent; the rats salivated copiously and coughed. There was a marked piloerection and severe diarrhoea and the rats felt cold and damp to the touch. In addition there was a loss of spontaneous motor activity, bradycardia and analgesia. The sedation increased in depth until the rats lost the righting reflex. Death usually occurred within 30 min of the loss of righting reflex. The effects of BYDL lasted for 6–8 h and survivors appeared to be normal 24 h after the injection.

Although pyrazole was able to prevent the behavioural effects of BDL, BEDL and BYDL, pretreatment with scopolamine at a dose sufficient to inhibit parasympathetic activity (1·5 mg kg^{-1} i.p.) did not affect the time course or outcome of the actions of BDL, BEDL or BYDL.

Hypothermic action of 2-butyne-1,4-diol. Since injections of BYDL caused rats to feel cold to the touch, the body temperature after BYDL was monitored in order to determine whether or not the drug produced hypothermia. The dose-response effects of BYDL on rat body temperature are shown in Fig. 1. Unless otherwise indicated the rats were maintained at room temperature (22°) throughout. Under these conditions control animals injected intraperitoneally with saline maintained a body temperature within the range 37·2°–37·9° over 5 h. A dose of 0·408 mmol kg^{-1} of BYDL produced a significant fall ($P < 0.02$) in body temperature after 2·5 h following the injection, this returned to normal within 12 h. Doses of 0·817 and 1·634 mmol kg^{-1} produced a rapid and prolonged fall in body temperature. The minimum temperature recorded was 30·1° 2·5 h after the injection of the higher dose. Rats given these higher doses did not survive beyond 4 h post-injection.

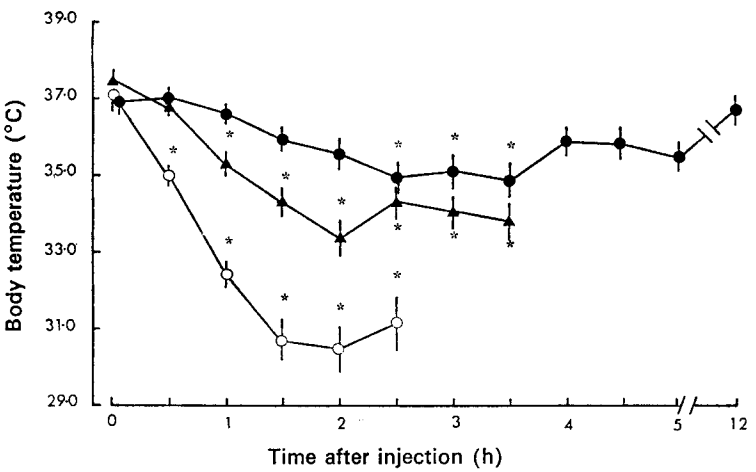

FIG. 1. Time course of the effects of varying doses of 2-butyne-1,4-diol (BYDL) on the body temperature of the rat. Rats were injected with BYDL at zero time as follows (mmol kg^{-1}, i.p.) ● 0·408; ▲ 0·817; ○ 1·634. The body temperature was determined at 30 min intervals by inserting a thermistor probe 4 cm into the rectum. Results are the means of at least 6 observations; the standard errors of the means (s.e.) are indicated by the vertical bars. Rats given 1·634 mmol kg^{-1} BYDL died between 2·5 and 3 h post-injection whilst those given 0·408 mmol kg^{-1} survived indefinitely. Points marked with an asterisk (*) are significantly lower ($P < 0.01$) than corresponding control values obtained with saline-injected rats.

To determine whether death was due to the profound fall in body temperature, the experiment was repeated with rats maintained at 32° (see Fig. 2). In this case, neither the elevated environmental temperature nor a dose of 1·5 mg kg^{-1} scopolamine 5 min before a dose of 0·817 mmol kg^{-1} BYDL significantly altered the time course or magnitude of the fall in body temperature from that observed in rats given BYDL alone (see Fig. 2). Also, the time at which death occurred was not altered by the scopolamine or high environmental temperature. On the other hand, the protective action of pyrazole can be clearly seen in Fig. 2. The mean body temperature of rats given 2·9 mmol kg^{-1} pyrazole 10 min before the BYDL never deviated significantly ($P > 0{\cdot}05$) from the corresponding control values obtained in saline-injected rats.

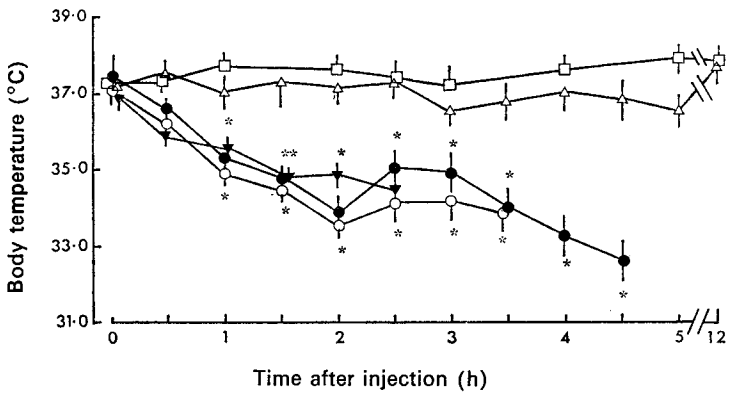

FIG. 2. Drug interactions with the hypothermic action of BYDL. Groups of 6 rats were injected (i.p.) as follows: □ 0·9% saline; ○ 70 mg kg^{-1} BYDL; ● 1·5 mg kg^{-1} scopolamine 5 min before BYDL; △ 225 mg kg^{-1} pyrazole 10 min before BYDL; ▲ 70 mg kg^{-1} BYDL; rats maintained at elevated environmental temperature (32°). Body temperatures were determined as described in Fig. 1. Results are means ± s.e. Points marked * are significantly lower ($P < 0{\cdot}02$) than corresponding control values. Those animals which survived 5 h after the injections (all the controls (□) and all the pyrazole + BYDL injected animals (△), had their temperature determined again at 12 h post-injection.

Effects of 2-butene-1,4-diol and butane-1,4-diol on rat body temperature. BEDL, at doses which produced marked sedation, produced no significant fall ($P > 0{\cdot}05$) in the rat body temperature (see Fig. 3). BDL, on the other hand, at a dose sufficient to produce loss of the righting reflex for 3 h also produced a significant fall ($P < 0{\cdot}02$) in the body temperature which correlated well with the duration of the sleeping time. However, the mean fall in body temperature (1·9°) was much less than that observed with BYDL (Fig. 1).

Effects of γ-butyrolactone and γ-hydroxybutyric acid on body temperature. Since there is now considerable evidence that BDL is, in itself, pharmacologically inactive and that GHB, a metabolite of BDL is the active principle, the effects of GHB and GBL (the cyclic form of GHB) on rat body temperature were also examined. GBL produced a very distinctive biphasic fall in body temperature following the injection of doses sufficient to produce loss of the righting reflex (Fig. 4A). At all three doses tested (200, 400 and 600 mg kg^{-1}) there was an initial fall in temperature followed after 2 h by a return to the control value or higher. After a short delay a second, more profound, fall in temperature occurred which lasted up to 4 h at the highest dose. All the animals had completely recovered by 12 h after the injection. The

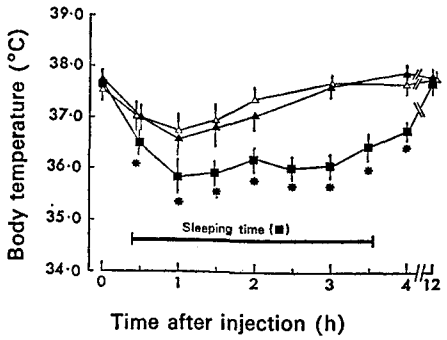

FIG. 3. Effects of butene-1,4-diol and butane-1,4-diol on rat body temperature. The body temperatures were determined as described in Fig. 1. The sleeping time was defined as the mean duration of the loss of righting reflex and is indicated by the horizontal broad line. Drugs were administered as follows (mg kg^{-1}, i.p.): ■ butane-1,4-diol 500; △ butene-1,4-diol 358; ▲ butene-1,4-diol 629. Results are the means ± s.e. for at least 6 observations. A significant ($P < 0.01$) fall in temperature from the control value is indicated by (*).

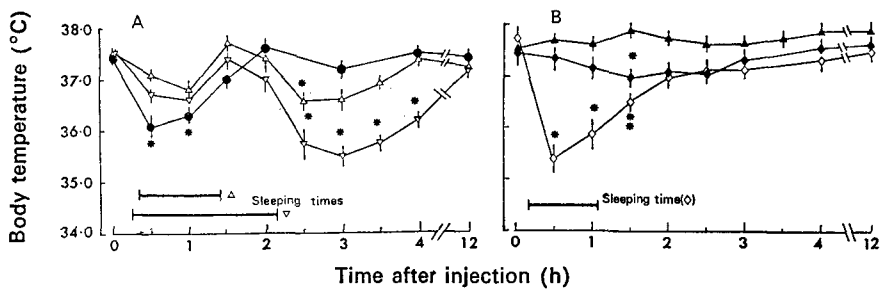

FIG. 4. Dose-response effect of (A) γ-butyrolactone (GBL) and (B) γ-hydroxybutyric acid (GHB) on rat body temperature. Groups of 6 rats were injected as follows (mg kg^{-1}, i.p.): A (GBL) ● 200; △ 400; ▽ 600; B ▲ 0·9% saline (control) ◆ 200; ◇ 400. The body temperatures were determined as described in Fig. 1. Sleeping time was defined as the mean duration of loss of the righting reflex. Points are the means ± s.e. (indicated by the vertical lines) and those significantly lower ($P < 0.02$) than the control values are indicated by (*).

sleeping times coincided with the first fall in temperature and the subsequent recovery, but at the highest dose the body temperature was still significantly lower ($P < 0.05$) than control values, although the rats had regained their righting reflex and were moving about freely. In contrast, with GHB at doses producing a loss of the righting reflex for about 60 min, only a single phase fall in body temperature occurred, and this did correspond with the period of the loss of the righting reflex (Fig. 4B). Sub-hypnotic doses of GHB produced no significant fall ($P > 0.05$) in body temperature. The mean fall in temperature observed following GBL or GHB was considerably less than that observed after BYDL: 1·5–2·0° compared to 7·2°.

To determine whether the fall in body temperature observed during GHB, GBL and BDL hypnosis was due to the loss of spontaneous motor activity, the effects of pentobarbitone in an anaesthetic (60 mg kg^{-1}) or hypnotic (30 mg kg) dose on body temperature were determined. The results are shown in Fig. 5. It is apparent that the significant fall in body temperature measured during pentobarbitone anaesthesia correlates closely with the duration of the sleeping time. Recovery from the anaesthetic at 2·5–3 h post-injection coincided with the return to normal body temperature.

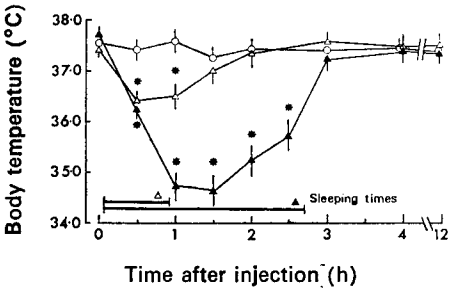

FIG. 5. Dose-response effect of pentobarbitone on rat body temperature. Groups of 6 rats were injected as follows (mg kg^{-1}, i.p.): ○ 0·9% saline (control); △ pentobarbitone, 30; ▲ 60. The body temperatures were determined as described in Fig. 1. Sleeping times were defined as the mean duration of the loss of the righting reflex. Points are the means ± s.e. and those significantly lower ($P < 0.02$) than the corresponding control values are indicated by (*).

Metabolism of 4-carbon diols by liver alcohol dehydrogenase. Since pyrazole, an inhibitor of alcohol dehydrogenase, was able to prevent the behavioural and toxic effects of all three diols tested, the ability of partially purified rat liver alcohol dehydrogenase (E.C.1.1.1.1.) to oxidize the diols was examined to determine whether an inhibition of alcohol dehydrogenase *in vivo* could explain this prophylactic action of pyrazole. The values for the apparent Michaelis constants for the oxidation of BDL, BEDL, and BYDL by alcohol dehydrogenase are: $1{\cdot}6 \times 10^{-3}$, $3{\cdot}4 \times 10^{-4}$, $8{\cdot}2 \times 10^{-4}$M respectively. Ethanol was $7{\cdot}9 \times 10^{-4}$M. It can be seen that all three diols were substrates for the enzyme and have apparent K_m values within the range $8{\cdot}2 \times 10^{-4}$ to $1{\cdot}6 \times 10^{-3}$M. Pyrazole competitively inhibited the oxidation of all 3 diols and the calculated values for K_I (obtained from the gradient of lines obtained by plotting reciprocal substrate concentration against reciprocal velocity) were between $5-15 \times 10^{-7}$M. Thus, pyrazole was a potent inhibitor of the metabolism of BDL, BEDL and BYDL by the rat liver extract.

DISCUSSION

The hypnotic effects of BDL have been well documented (Sprince & others, 1966; Gessa, Spano & others, 1968; Taberner & others, 1972) although there is now overwhelming evidence that BDL is, in itself, inactive and is converted *in vivo* by liver alcohol dehydrogenase and aldehyde dehydrogenase to GHB (Kerkut & Taberner, 1971; Bessman & McCabe, 1972; Maxwell & Roth, 1972). Similarly, it is now accepted that GBL is inactive and is converted by a lactonase in the plasma to GHB (Giarman & Roth, 1964; Roth & Giarman, 1965). In the present work, GHB, GBL and BDL produced similar behavioural effects although there was a considerable latency between the injection and the onset of the hypnosis in the case of BDL. The behavioural effects and toxicity of BEDL and BYDL have not previously been fully described, but the brief observations of Sprince & others (1966) that BYDL produced loss of spontaneous activity and severe diarrhoea in rats have been confirmed in the present work, and it has been shown that BYDL is more toxic than BEDL which, in turn, is more toxic than BDL (Table 1).

The prevention of the toxic effects of BEDL and BYDL by the inhibition *in vivo* of alcohol dehydrogenase by pyrazole implies that it is the products of the oxidative metabolism of BEDL and BYDL which are responsible for the toxicity of these diols. The inhibition by pyrazole of the oxidation of BEDL and BYDL by rat liver extract

in vitro supports this hypothesis which is similar to that previously proposed to explain the interaction of pyrazole with BDL (Bessman & McCabe, 1972; Taberner & others, 1972). Intracerebral injection of BEDL and BYDL would provide direct evidence for this hypothesis since the brain level of alcohol dehydrogenase is not sufficient to oxidize an exogenous alcohol significantly (Raskin & Sokoloff, 1970; Taberner, 1974).

Although the hypothermia following BYDL was far more profound than that observed after BDL, GBL or GHB, the experiments with rats maintained at 32° (Fig. 2) imply that the hypothermia in itself was not the cause of death. Reviews of the effects of drugs on body temperature (Cremer, 1969; Lomax, 1970) indicate that most depressant drugs, at doses which inhibit spontaneous activity, produce a fall in body temperature, probably due to the fall in heat production from the activity of voluntary muscles. In the present work, a fall in body temperature of 1·0–2·0° occurred during the period of the loss of righting reflex induced by BDL, GBL, GHB and pentobarbitone. This fall can be considered to be the result of the depressant action of the drugs, although a direct central hypothermic action cannot be ruled out (see Lomax, 1966). The 7·0° fall in temperature following BYDL (Fig. 1) is thus due to additional factors. Parasympathomimetic agonists have been shown to produce hypothermia (Friedman & Jaffe, 1969) presumably due to a central action on the thermoregulatory centres of the anterior hypothalamus (Bligh & Cremer, 1969). The central hypothermic action of a parasympathetic agonist can be blocked by scopolamine (Friedman & Jaffe, 1969), but, in the present work, pretreatment with scopolamine had no effect on the time course of the hypothermic response to BYDL therefore it is probably not acting by the same mechanism.

Although the hypothermic responses to BDL and GHB correlated with the sleeping time both in terms of onset and duration, following GBL there was a biphasic response. The second fall in temperature occurred after the animals had returned to normal behaviour. This observation confirms that of Borbely & Huston (1972) and suggests that GBL itself may have long-term hypothermic actions unrelated to the initial hypothermic response which coincides with the behavioural depression.

BYDL is finding increasing uses in industry, but there have been no detailed reports of its behavioural effect or toxicity. Also, although it is readily available from commercial sources, there are no health hazard warnings displayed on the containers in which it is supplied. Warnings of possible toxicity are given with other, less toxic, diols such as ethylene glycol and, in this light, more detailed studies of the toxicity of BYDL should certainly be made.

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