Br. J. Pharmac. (1975), **54**, 171-179

ALTERNATIVE APPROACHES TO ANALGESIA: BACLOFEN AS A MODEL COMPOUND

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1 It is suggested that analgesia could be produced by drug action at the spinal level through (a) interference with neurotransmission at primary afferent terminals; (b) enhancement of the 'gate control' of the sensory input to the spinal cord mediated through descending spinal tracts; or (c) increased presynaptic inhibition of primary afferents by a direct action.

2 Baclofen (9.4-70.3 μmol/kg, i.p.), which may mimic spinal presynaptic inhibition, produced a dose-dependent increase in the response times of mice in a hot-plate test, but high doses also impaired motor function.

3 Morphine hydrochloride (5.3-40 μmol/kg, i.p.) increased the response time of mice in the hot-plate test and had little effect on motor function.

4 Combination of baclofen (9.4 or 23.4 μmol/kg) with morphine (13.3 μmol/kg) produced greater increases in response time than either drug administered alone but with little concurrent effect on motor function.

5 The possibility that baclofen may have some analgesic action and a potentiating effect on other analgesics is discussed.

Introduction

The major classes of analgesic agents in current clinical use have a number of drawbacks. The narcotic analgesics, although potent, have several undesirable actions and continued use may lead to dependence. This latter hazard is not encountered with the antipyretic analgesics but these agents are generally more limited in terms of analgesic potency. Structural modification of the narcotic analgesics has produced some advances but an alternative approach to the design of analgesics would be an attractive proposition and may prove to be more fruitful. One such approach would be to modify the sensory input to the central nervous system at the spinal level thereby reducing the transmission of information related to a painful stimulus to higher centres. This approach would clearly present a problem of selectivity of drug action but three possible mechanisms of action might be considered:

(1) Interference with neurotransmission at primary afferent terminals

The logical development of drugs having a selective action at sites in the spinal cord almost certainly depends on the identification of the neurotransmitters involved in sensory pathways and the associated synaptic events. The substance which is currently most favoured as a candidate for a neurotransmitter released by primary afferent terminals is glutamic acid (Hammerschlag & Weinreich, 1972; Johnson, 1972) although the evidence is not yet convincing and this amino acid has not been specifically linked with one or more groups of primary afferent fibres. An additional candidate is the polypeptide Substance P which is found in high concentrations in dorsal roots and has more powerful excitatory effects on spinal neurones than glutamic acid (Otsuka, Konishi & Takahashi, 1972a,b; Konishi & Otsuka, 1974).

If either of these compounds in time proves to be an excitatory neurotransmitter released by primary afferent neurones which convey pain-related information, then the discovery of drugs which reduce the postsynaptic availability or postsynaptic interaction of the transmitter might constitute an important advance in the development of alternative analgesics. The finding of Haldeman & McLennan (1972) that glutamic acid diethylester blocks the excitatory action of iontophoretically applied glutamic acid and also synaptic activation of dorsal horn interneurones in the cat spinal cord and in the thalamus (Haldeman, Huffman, Marshall & McLennan, 1972) is an interesting development in this respect.

(2) Enhancement of the 'gate control' of the afferent input to the spinal cord mediated through descending spinal tracts

Melzack & Wall (1965) have suggested that the afferent impulses which trigger pain reactions pass through a gate control mechanism whose effectiveness is determined by a balance of the activity in large and small cutaneous afferent fibres and by impulses descending from higher centres. The gating control is believed to be operated through presynaptic inhibition of primary afferent terminals and has been shown to be particularly effective in reducing the activity of receptive interneurones of (Rexed, 1952) laminae IV and V (Wall, 1967; Hillman & Wall, 1969). There is accumulating evidence to indicate that morphine analgesia is in part associated with an increase in the activity of tryptaminergic pathways descending from the dorsal raphe to inhibit the discharge of lamina IV and V cells in the spinal cord (Vogt, 1974). Stimulation of the dorsal raphe produces profound analgesia in anaesthetized or conscious cats (Liebeskind, Guilbaud, Besson & Oliveras, 1973; Oliveras, Besson, Guilbaud & Liebeskind, 1974) and a similar response in rats was reduced by the administration of *p*-cholorophenylalanine (Akel, Mayers & Liebeskind, 1972).

Increasing by pharmacological means the postsynaptic availability of the presumed neurotransmitter, 5-hydroxytryptamine, in the descending control of the spinal gating mechanism could therefore be an alternative approach to analgesia.

(3) Increase of presynaptic inhibition of afferent neurones by a direct action

There is considerable experimental support for the suggestion that γ-aminobutyric acid (GABA) is a neurotransmitter released by neurones which mediate presynaptic control of primary afferent terminals. Spinal GABA concentrations are highest in dorsal horn (Graham, Shank, Werman & Aprison, 1967; Johnston, 1968) and levels are reduced by chronic dorsal root section which suggests a close association of GABA with primary afferent terminals (Gottesfeld, Kelly & Rayner, 1973; Jones, Jordan, Morton, Stagg & Webster, 1974). Furthermore, presynaptic inhibition (inferred from dorsal root potentials) initiated from inputs to various levels of the cat central nervous system (CNS) is antagonized by bicuculline (Benoist, Besson, Conseiller & Le Bars, 1972; Benoist, Besson & Boissier, 1974) which also blocks the effects of GABA on spinal neurones (Curtis, Duggan, Felix & Johnston, 1971). Levy & Anderson (1974) have additionally suggested that primary afferent hyperpolarization may be GABA-mediated since it may be blocked by bicuculline and picrotoxin. GABA has been shown to depolarize primary afferent terminals thereby mimicking presynaptic inhibition (Eccles, Schmidt & Willis, 1962; 1963; Barker & Nicoll, 1972; Levy, 1974; Nishi, Minota & Karczmar, 1974) although these studies have concentrated on the presynaptic control of monosynaptic reflexes. However, if GABA has a similar role in the control of cutaneous afferents, then an analgesic effect might be achieved by increasing the availability of GABA (or related compound) at receptors on afferent terminals.

GABA does not itself cross the blood-brain barrier but the *p*-chlorophenyl substituted derivative (baclofen) readily does so and is used clinically for the treatment of spasticity (Lioresal, CIBA). There is evidence to suggest that it may reduce sensory input to the cord by mimicking presynaptic inhibition (Pierau & Zimmerman, 1973) although this view has been questioned by Curtis, Game, Johnston & McCulloch (1974).

The possibility remains that if baclofen were found to mimic presynaptic inhibition of the afferent input of pain-related information, then this compound might have analgesic properties. The results of clinical trials on patients suffering from spastic conditions certainly indicate that the drug afforded considerable relief from the pain which accompanied the condition (Pinto, Polikar & Debono, 1972). However, it is difficult to determine whether this effect is solely the result of reduced muscle contracture.

Since baclofen is apparently relatively specific to the spinal cord in its action, (Koella, 1972) it was an obvious candidate for initial investigations towards possible alternative analgesic agents. We have therefore studied the effect of baclofen on the response of mice to a noxious (thermal) stimulus and compared the effects of this drug with those of morphine. Since animal tests can show only whether the response of the subject to a noxious stimulus is altered by drug treatment, we prefer to use the term antinocisponsive action rather than analgesic or antinociceptive action to describe a drug-induced delay of the response.

Methods

Experiments were performed on male Swiss Albino mice weighing between 25 and 30 g. They were moved to the laboratory at least one hour before starting the experiment.

Antinocisponsive test

Each test was performed as follows. The mouse was lifted by the tail and placed on a hot plate (55°C ± 0.5°C) and covered by a 250 ml glass beaker. A stop watch was started as the hind paws made contact with the hot-plate and stopped as soon as an end-point was observed, at which time the animal was removed and the response time recorded in hundredths of 1 minute. Two behavioural end-points were accepted. The first was shaking of one or both hind paws and the second was bringing the hind paw into contact with the mouth. If an end-point had not been reached after 0.60 min (the 'cut-off point'), the animal was removed from the hot-plate to prevent damage to the paws. The use of a beaker to cover the animal ensured that the hind paws were in continuous contact with the hot-plate since the animal's normal reaction to this environment was to rear up against the wall of the beaker. This method was found to give consistent end-points in pilot experiments. A control reading was taken for each animal before drug treatment and then at 15 or 30 min intervals after drug treatment.

Tests of motor function

Motor function was tested in each animal by placing it on a horizontal wooden bar (2.5 cm diameter) which rotated at 20 rev minute^{-1}. An untreated mouse when placed on the upper aspect of the bar was able to remain in this position by means of forward locomotion for a period of 1 minute. A mouse with impaired motor co-ordination was unable to maintain this position and in an extreme case, it would fall off the bar. Intermediate cases, however, would turn passively with the bar. The number of times that an animal rotated with the bar was used as the basis for the first test of motor function. The duration, d, of the test (normally 1 min) was recorded together with r the number of times the animal rotated passively with the bar. Since the bar rotation rate was 20 rev min^{-1}, the maximum number of passive rotations that a mouse could undergo was 20. The score assigned to each animal was given by $20 - r$. Occasionally, an animal would fall off the bar in less than one minute. In such a case, a second test was conducted and frequently a value for d of 1 min was achieved. On the few occasions where the mouse failed to remain on the bar for 1 min in the second test, r was computed from $r \times 1/d$ and this value was used to compute the score for that animal. Changes in performance following drug treatment are expressed as Δp (= mean post injection score − mean pre-injection score). A positive value indicates an improved performance and a negative value a deterioration.

A second test was applied immediately after the first. If a normal mouse was reorientated on the bar such that it would have to walk backwards in order to maintain its position on the upper aspect of the bar, it would simply return to its former orientation. The ability of treated and untreated mice to perform this manoeuvre was scored on a simple pass or fail scheme.

These tests were performed on each animal before drug treatment and at 30 min intervals thereafter. In addition to the standardized tests each animal was closely observed for changes in behaviour.

Drug treatment

Drug treatments were assigned on a random basis within each group of animals. Each animal was weighed immediately before the start of the experiment and all drug concentrations were such that the injection volume was 10 ml/kg. Saline (0.9% w/v NaCl solution) controls were administered on a similar basis. The experiments were performed 'blind' in that the experimenter was not aware of the drug treatment given to individual animals.

Drugs were dissolved in 154 mM sodium chloride and administered intraperitoneally. Doses of morphine hydrochloride and baclofen (Lioresal, CIBA) are stated as μmol/kg.

Results

Antinocisponsive effects of morphine and baclofen

Four dose levels of each drug were tested and both morphine and baclofen were found to increase the response time for mice in the hot-plate test. Examples of results for two treatment groups are illustrated in graphical form in Figure 1. The time dependence of response time in each treatment group was tested by Friedman two way analysis of variance (Null hypothesis – the reaction times of the mice receiving a given treatment were independent of the time at which they were tested). Probabilities derived from this test are shown in Table 1. In five of the drug treatments the responses varied significantly ($P < 0.05$) with time but for the lowest dose of each drug, the null hypothesis could be rejected with less certainty and similarly for 26.6 μmol/kg morphine. Analysis of the results for saline-treated animals showed that the reaction times were independent of the time at which they were measured and the

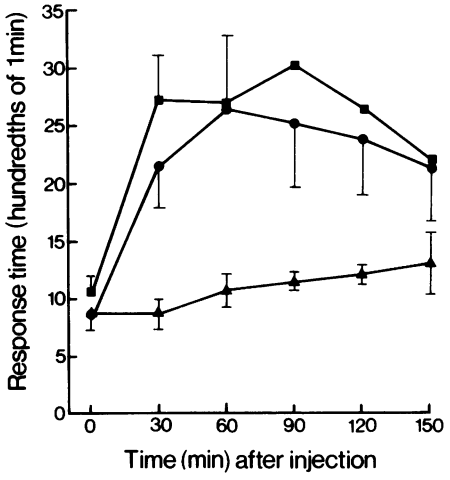

Figure 1 Reaction times of mice in hot-plate test determined at 30 min intervals. The first point on each curve is the pre-injection control reading which served as the lower limit for the determination of 'total effects' (see text). (■) Morphine, 26.6 μmol/kg; (●) baclofen, 23.4 μmol/kg; (▲) saline, 10 ml/kg. Each point is the mean result from 6 animals. Standard errors are not included where one or more members of the group reached the cut-off point.

apparent upward trend of the response (Figure 1) was not significant.

The antinocisponsive effects of the two drugs have been computed by two methods:

(a) The total antinocisponsive effect for the pooled results from each group was determined as follows. The response time (in hundredths of 1 min) was plotted against time (in min) after administration of the drug as shown in Figure 1.

Table 1 Probability that the reaction times in the hot-plate test measured throughout the course of the experiment are independent of the time at which they were measured.

Drug	*Dose (μmol/kg)*	P
Morphine	5.3	< 0.20
	13.3	< 0.05
	26.6	< 0.10
	40.0	< 0.01
Baclofen	9.4	< 0.20
	23.4	< 0.05
	46.9	< 0.05
	70.3	< 0.02
Saline	10.0 (ml/kg)	< 0.50

Results for each treatment group of 6 animals were analyzed by the Friedman Two-Way Analysis of Variance.

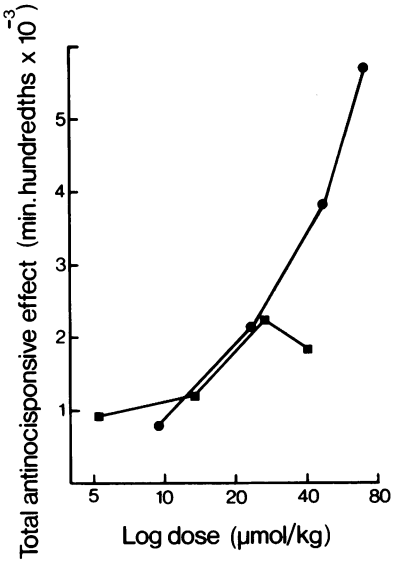

Figure 2 Log dose-response curves for the total antinocisponsive effects of baclofen (●) and morphine (■). Each point is the mean result from 6 animals. Results, when analyzed by the Kruskall-Wallis one-way analysis of variance (Null hypothesis – there is no difference between total effects of the four dose levels of morphine) showed no significant ($P < 0.05$) difference ($P < 0.1$). However, if the lowest dose is omitted from the analysis, $P < 0.05$. Equivalent value for the four doses of baclofen is $P < 0.001$).

The area delineated by the graph and a horizontal line drawn from the pre-injection control response was determined and this value (expressed in min hundredths) was used as an index of the total antinocisponsive effect. This method is analogous to that of Winter & Flataker (1950).

Log dose-response curves constructed on the basis of calculated total effects are shown in Figure 2. Both drugs produced dose-dependent increases in the reaction time although the highest dose of morphine (40 μmol/kg) appeared to be less effective than 26.6 μmol/kg. The antinocisponsive effect of baclofen consistently increased with dose and the two higher doses produced greater total effects than any dose of morphine.

(b) Peak effects – since the time course of action of the two drugs varied and the onset of effects in individual animals was somewhat variable, the measured peak effects of the treatments occurred at different times. Peak effects for individual animals have therefore been determined and the mean for each treatment group plotted as log dose-response curves for the two drugs, (Figure 3). The curve for morphine again indicates

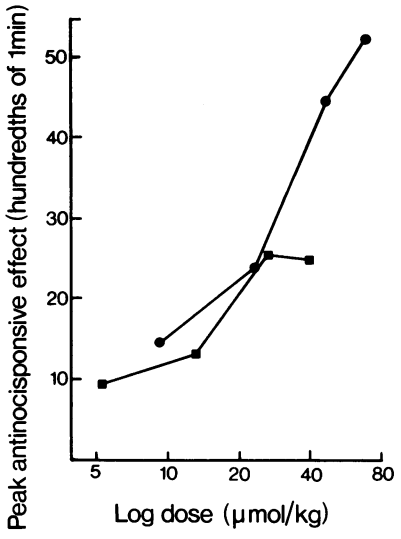

Figure 3 Log dose-response curves for the peak antinocisponsive effects of morphine (■) and baclofen (●). Kruskall-Wallis analysis of the full dose range of each drug yields $P < 0.02$ and $P < 0.001$ for morphine and baclofen respectively. Each point is the mean from 6 animals.

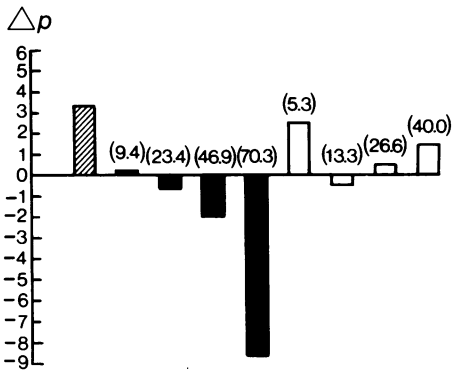

Figure 4 Performance of mice in rotation test of motor function. A positive index of performance Δp, indicates improvement relative to the pre-injection control score. A negative value of Δp indicates a deterioration. Figures in brackets are doses in μmol/kg. Each column is the mean score of a treatment group of 6 animals. Hatched columns — saline (10 ml/kg); solid columns — baclofen; open columns — morphine.

that the response reaches a maximum at the 26.6 μmol/kg dose level. In contrast, baclofen produced a dose-dependent increase in peak effect, throughout the dose range.

Motor function tests

Motor function as denoted by the performance of each treatment group in the rotation test is shown in Figure 4. The highest dose of baclofen (70.3 μmol/kg) produced a severe depression of performance in this test. Lower doses had a less marked effect but only the lowest dose (9.4 μmol/kg) gave a positive index of performance (Δp). Positive values of Δp were obtained for all morphine treatments except for the 13.3 μmol/kg dose which slightly impaired performance. All drug treatments resulted in Δp values less than that obtained for saline controls.

In the reorientation test, only two drug treatments produced a marked change in performance relative to saline controls, most scores remaining near the 100% level. Baclofen 70.3 μmol/kg impaired performance such that re-orientation was successfully accomplished in only 35% of 36 attempts, whereas 46.9 μmol/kg baclofen reduced the score to 76%.

It was found repeatedly that the two highest doses of baclofen were associated with exophthalmos and piloerection which suggests that the drug may have a direct or indirect action on the autonomic nervous system. In addition, these two treatments often reduced spontaneous activity and in some animals receiving the highest dose there was a degree of catalepsy. Some of these animals could be rolled on to their dorsal surface and after a few seconds in this position, they would suddenly right themselves.

Effects of mixtures of baclofen and morphine

Since low doses of baclofen apparently had an antinocisponsive action without obvious impairment of motor function, it seemed worthwhile to determine whether a more powerful antinocisponsive effect could be achieved by the administration of a low dose (9.4 or 23.4 μmol/kg) of baclofen together with a low dose of morphine (13.3 μmol/kg). The effect of administration of these mixtures was compared with that of the individual doses administered alone and the usual saline controls. Log dose-response curves based on total antinocisponsive effects are shown in Figure 5. The effects of the mixture were found to be greater than those observed with individual doses of either drug in this experimental series. Furthermore, the 23.4 μmol/kg baclofen plus 13.3 μmol/kg morphine mixture produced a greater effect than any single dose of morphine in the first experimental series. The antinocisponsive effect of the two drugs administered as a mixture

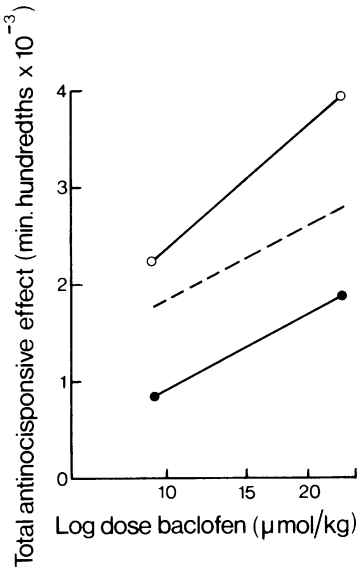

Figure 5 Log dose-response curves for baclofen alone (•) and in combination with morphine 13.3 μmol/kg (○). The dotted line indicates the theoretical curve derived from addition of the total effects of baclofen and morphine administered as separate treatments. Each point is the mean total antinocisponsive effect of groups of six animals tested at 15 min intervals over a period of 150 minutes. Statistical analysis, (Kruskall-Wallis) showed that combination of morphine with baclofen produced an increase in the effect relative to baclofen alone. The higher dose of mixture or of baclofen alone produced a greater effect than the corresponding lower doses ($P < 0.01$ in all cases).

thus appears to be at least additive. In fact, if the dose-response relationship for the mixtures is compared with that predicted from simple addition of the total effects for baclofen and morphine administered alone (dotted line in Figure 5) there is a suggestion of potentiation.

Log dose-response curves derived from peak effects in individual mice confirm the additive effects of the two drugs when administered as a mixture but 'potentiation' is apparent only at the lower dose level. This discrepancy is due to the fact that four of the animals that received the higher dose of mixture reached the cut-off point in the hot-plate test without giving a definite end-point. The cut-off point is more limiting in peak effect than in total effect determinations since the latter are based on responses monitored over a period of 150 min whereas peak effects are calculated from single observations, the maximum value of which is pre-set.

A striking feature of the effects of the mixtures

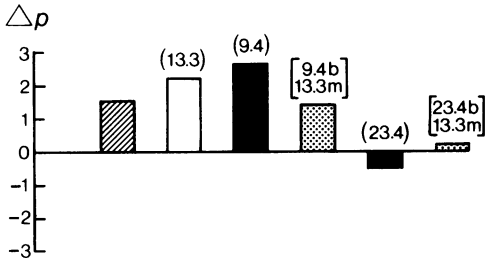

Figure 6 Rotation test performance for mice treated with mixtures or morphine or baclofen alone. Indices of performance expressed as for Figure 4. Hatched columns – saline (10 ml/kg); open columns – morphine; solid columns – baclofen; dotted columns – baclofen (b) plus morphine (m).

is that they produce marked antinocisponsive actions but neither dose caused a deterioration in performance in the rotation test. In fact, both mixture treatments were associated with small positive Δp values.

A similar pattern was reflected in the re-orientation test where both doses of mixture were without effect relative to saline.

All mice in this second experimental series seemed normally active and coordinated from subjective assessment. Thus, in contrast to some of the mice that received high doses of baclofen in the first experimental series, there was no obvious disability to which increased reaction times in the hot-plate tests could be attributed. However, it should be noted that during testing on the hot-plate, some animals treated with a mixture were apparently distressed by the environment in that they made attempts to escape from the confines of the beaker although they had not given a recognised end-point in the test. In control observations of these animals with the plate removed from the heat source this behaviour was not seen. This suggests that the observed distress response was associated in some way with the perception of heat (or possibly humidity above the water bath) although, as already indicated, there was nothing to suggest that the animals were unable to respond normally in the hot-plate test.

Discussion

The aim of this study was to determine whether baclofen has an antinocisponsive effect which might be related to an analgesic action. The determination of analgesic effects in animals is a difficult procedure since it is necessary to discriminate between an inability to respond to a

noxious stimulus and a genuine analgesia. We have therefore attempted to determine the effects of morphine and baclofen on motor function in mice by means of two standardized tests together with subjective assessment of behaviour (albeit in 'blind' trials). The large increases in reaction times in the hot-plate test observed with 46.9 and 70.3 μmol/kg baclofen were associated with a deterioration in performance in the motor function tests. The apparent 'analgesic' effect of these two treatments was therefore probably due to a reduced ability to execute an end-point response. Tests with animals treated with lower doses of baclofen (9.4 and 23.4 μmol/kg) did not reveal any marked deterioration in performance although the possibility remains that the motor function tests were not sufficiently sensitive to detect subtle changes. However, none of the animals treated with these low doses of baclofen showed any of the cataleptic or other behavioural effects seen at higher dose levels. Also, animals receiving low doses generally gave clear end-point responses in the hot-plate test which gave the impression that it may be possible to obtain some selectivity of action towards antagonism of perception of or response to a noxious stimulus without markedly affecting motor function.

The important difference between the effects of the drug combinations and those of baclofen administered alone is that the combinations afforded a powerful antinocisponsive action without a decrease in performance in the motor function tests, although our reservation that some animals showed some distress during the hot-plate test should be reiterated.

This study was based on the premise that an increase in presynaptic inhibitory control over sensory inputs to the spinal cord would result in an analgesic effect. What evidence is there to suggest that the marked antinocisponsive action is mediated through presynaptic inhibition? The possibility that GABA is a presynaptic inhibitory neurotransmitter implies that an increased availability of GABA at receptors on primary afferent neurones would damp down the sensory input. Pierau & Zimmerman (1973) have provided indirect evidence from intracellular recording of motoneurones that intravenously administered baclofen mimics presynaptic inhibition in the spinal cord. This suggestion has recently been challenged by Curtis *et al.*, (1974) since they were unable to demonstrate antagonism by biculline of the inhibitory effects of iontophoretically applied baclofen on spinal neurones. Bicuculline has previously been shown to antagonize presynaptic inhibition (Levy, Repkin & Anderson, 1971; Benoist *et al.*, 1972) and the effects of GABA in the cat spinal cord (Curtis *et al.*, 1971). Curtis *et al.* (1974) found that the uptake of 10^{-8}M L-glutamic acid into brain slices was reduced by 34% in the presence of baclofen (10^{-4}M) and that 'GABA transaminase activity' was reduced by 35% in the presence of 10^{-3}M baclofen. On the basis of these observations, they suggest that of the GABA receptors present in the CNS, baclofen possibly only interacts with those of GABA transaminase and the reduction *in vitro* of high affinity L-glutamic acid uptake does not account for the depression of neuronal firing by baclofen. However, estimations based on the data of Faigle & Keberle (1972) suggest that the CNS concentrations of baclofen achieved in rats after intravenous administration of a high dose (47 μmol/kg) are of the order of 5×10^{-6}M. It seems unlikely therefore that the pharmacological action of baclofen is primarily the result of inhibition of GABA transaminase or of glutamic acid uptake.

We find that the dosage of baclofen is extremely critical. Effects may vary from slight to severe depression of reflexes with concurrent respiratory depression or even, in some cases, a paradoxical phase of hyperirritability, all occurring within a relatively small dose range. It is difficult therefore to relate the CNS concentrations associated with given behavioural effects with those encountered locally during iontophoretic application of baclofen. If presynaptic inhibitory connections are the site of action of intravenously administered baclofen as suggested by Pierau & Zimmerman (1973), it is important to determine whether primary afferent depolarization is altered by this compound and whether the synaptic activation of dorsal horn interneurones is modified in a fashion identical with that normally associated with increased presynaptic inhibition of pain related afferent input to the spinal cord.

Selectivity of action is a fundamental requirement for an analgesic having a spinal locus of action. Although baclofen is unlikely to prove to be an ideal analgesic, its relative specificity to the spinal cord (Koella, 1972) and its potent antinocisponsive action, particularly in combination with morphine, render it worthy of further study. Even if it is not an analgesic in doses which do not produce depression of spinal reflexes it might be of use as an adjunct to other analgesics or in certain aspects of spinal anaesthesia.

We feel that analgesic action at a spinal level is a useful approach and we would welcome the development of compounds which may be utilized in this way.

We are indebted to Mr F.W. Ballhatchet for his construction of the apparatus, to Dr A.M. Jukes (Medical Adviser, C.I.B.A. Laboratories) for the provision of baclofen, and to Dr R.A. Webster for his critical appraisal of the manuscript.

References

AKIL, H., MAYERS, D.J. & LIEBESKIND, J.C. (1972). Comparaison chez le rat entre l'analgésie induite par stimulation de la substance grise péri-aquéducale et l'analgésie morphinique. *C.r. hebd. Séanc. Acad. Sci. Paris,* **274**, 3603-3605.

BARKER, J.L. & NICOLL, R.A. (1972). Gamma-aminobutyric acid: role in primary afferent depolarization. *Science,* **176**, 1043-1045.

BENOIST, J.M., BESSON, J.M. & BOISSIER, J.R. (1974). Modifications of presynaptic inhibition of various origins by local application of convulsant drugs on cat's spinal cord. *Brain Research,* **71**, 172-177.

BENOIST, J.M., BESSON, J.M., CONSEILLER, C. & LE BARS, D. (1972). Action of bicuculline on presynaptic inhibition of various origins in the cat's spinal cord. *Brain Research,* **43**, 672-676.

CURTIS, D.R., DUGGAN, A.W., FELIX, D. & JOHNSTON, G.A.R. (1971). Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord of the cat. *Brain Research,* **32**, 69-96.

CURTIS, D.R., GAME, C.J., JOHNSTON, G.A.R. & McCULLOCH, R.M. (1974). Central effects of beta-(*p*-chlorophenyl)-gamma aminobutyric acid. *Brain Research,* **70**, 493-499.

ECCLES, J.C., SCHMIDT, R.F. & WILLIS, W.D. (1962). Presynaptic inhibition of the spinal monosynaptic reflex pathway. *J. Physiol., Lond.,* **161**, 282-297.

ECCLES, J.C., SCHMIDT, R.F. & WILLIS, W.D. (1963). Pharmacological studies on presynaptic inhibition. *J. Physiol., Lond.,* **168**, 500-530.

FAIGLE, J.W. & KEBERLE, H. (1972). The chemistry and kinetics of Lioresal. *Postgrad. med. J.,* **48**, Suppl. 5.

GOTTESFELD, Z., KELLY, J.S. & RAYNER, C.N. (1973). Depletion of glutamic acid decarboxylase (GADI) activity from the spinal cord following dorsal-root section. *J. Physiol., Lond.,* **231**, 25-26P.

GRAHAM, L.T., SHANK, R.P., WERMAN, R. & APRISON, M.H. (1967). Distribution of some synaptic transmitter suspects in cat spinal cord: glutamic acid, aspartic acid, γ-aminobutyric acid, glycine and glutamine. *J. Neurochem.,* **14**, 465-472.

HALDEMAN, S., HUFFMAN, R.D., MARSHALL, K.C. & McLENNAN, H. (1972). The antagonism of the glutamate-induced and synaptic excitation of thalamic neurones. *Brain Research,* **39**, 419-425.

HALDEMAN, S. & McLENNAN, H. (1972). The antagonistic action of glutamic acid diethylester towards amino acid-induced and synaptic excitations of central neurones. *Brain Research,* **45**, 393-400.

HAMMERSCHLAG, R. & WEINREICH, D. (1972). Glutamic acid and primary afferent transmission. *Adv. Bioch. Psychopharmac.,* **6**, 165-180.

HILLMAN, P. & WALL, P.D. (1969). Inhibitory and excitatory factors influencing the receptive fields of lamina 5 spinal cord cells. *Exp. Brain Res.,* **9**, 284-306.

JOHNSON, J.L. (1972). Glutamic acid as a synaptic transmitter: a review. *Brain Research,* **37**, 1-19.

JOHNSTON, G.A.R. (1968). The intraspinal distribution of some depressant amino acids. *J. Neurochem.,* **15**, 1013-1017.

JONES, I.M., JORDAN, C.C., MORTON, I.K.M., STAGG, C.J. & WEBSTER, R.A. (1974). The effect of chronic dorsal root section on the concentration of free amino acids in the rabbit spinal cord. *J. Neurochem.,* **23**, 1239-1244.

KOELLA, W.P. (1972). Pharmacological aspects of spasticity with special reference to Lioresal. *Postgrad. med. J.,* **48**, Suppl. 5, 13-18.

KONISHI, S. & OTSUKA, M. (1974). The effects of Substance P and other peptides on spinal neurons of the frog. *Brain Research,* **65**, 397-410.

LEVY, R.A. (1974). GABA: a direct depolarizing action at the mammalian primary afferent terminal. *Brain Research,* **76**, 155-160.

LEVY, R.A. & ANDERSON, E.G. (1974). The role of γ-aminobutyric acid as a mediator of positive dorsal root potentials. *Brain Research,* **76**, 71-82.

LEVY, R.A., REPKIN, A.H. & ANDERSON, E.G. (1971). The effect of bicuculline on primary afferent terminal excitability. *Brain Research,* **32**, 261-265.

LIEBESKIND, J.C., GUILBAUD, G., BESSON, J.M. & OLIVERAS, J-L. (1973). Analgesia from electrical stimulation of the periaqueductal gray matter in the cat: behavioural observations and inhibitory effects on the spinal cord interneurones. *Brain Research,* **50**, 441-446.

MELZACK, R. & WALL, P.D. (1965). Pain mechanisms: a new theory. *Science,* **150**, 971-979.

NISHI, S., MINOTA, S. & KARCZMAR, A.G. (1974). Primary afferent neurones: the ionic mechanism of GABA – mediated depolarization. *Neuropharmacology,* **13**, 215-219.

OLIVERAS, J.L., BESSON, J.M., GUILBAUD, G. & LIEBESKIND, J.C. (1974). Behavioural and electrophysiological evidence of pain inhibition from mid brain stimulation in the cat. *Exp. Brain Res.,* **20**, 32-44.

OTSUKA, M., KONISHI, S. & TAKAHASHI, T. (1972a). The presence of a motoneuron-depolarizing peptide in bovine dorsal roots of spinal nerves. *Proc. Japan Acad.,* **48**, 342-346.

OTSUKA, M., KONISHI, S. & TAKAHASHI, T. (1972b). A further study of the motoneuron-depolarizing peptide extracted from dorsal roots of bovine spinal nerves. *Proc. Japan. Acad.,* **48**, 747-752.

PIERAU, F.K. & ZIMMERMAN, P. (1973). Action of a GABA-derivative on postsynaptic potentials and membrane properties of cat's spinal motoneurones. *Brain Research,* **54**, 376-380.

PINTO, O. de S., POLIKAR, M. & DEBONO, G. (1972). Results of international clinical trials with Lioresal. *Postgrad. med. J.,* **48**, Suppl. 5, 18-23.

REXED, B. (1952). The cytoarchitectonic organization of the spinal cord of a cat. *J. cell. comp. Neurol.,* **96**, 415-495.

VOGT, M. (1974). The effect of lowering the 5-hydroxytryptamine content of the rat spinal cord on analgesia produced by morphine. *J. Physiol., Lond.,* **236**, 483-498.

WALL, P.D. (1967). The laminar organization of dorsal horn and effects of descending impulses. *J. Physiol., Lond.,* **188**, 403-423.

WINTER, C.A. & FLATAKER, L. (1950). Studies on heptazone (6-morpholino-4,4-diphenyl-3 heptanone hydrochloride) in comparison with other analgesic drugs. *J. Pharmac. exp. Ther.*, **98**, 305-316.

(Received September 30, 1974)